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Description

The invention relates generally to the field of receptor binding assays for analytes in samples suspected of containing the analyte. One problem associated with receptor binding assays for analytes is that samples containing the analytes may contain other materials that will bind receptor reagents utilized in an assay. For example, the interfering material can bind to an antibody which is utilized in the assay, thus reducing the amount of antibody available for binding to an analyte. Another problem is that some analytes bind to interfering materials present in the sample and become complexed. In a complex form the analyte is unavailable for binding to receptors used in the assay and therefore an accurate assay for such analyte is difficult to achieve. Consequently, it becomes necessary to disrupt the binding of the analyte with the interfering material prior to or simultaneously with conducting the assay for the analyte without disrupting binding of the analyte to the receptor.

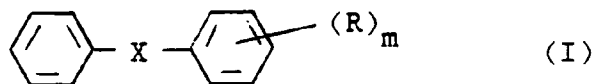
Urine samples from individuals taking certain non-steroidal, anti-inflammatory drugs such as fenoprofen contain a substance that cross reacts with a subset of the polyclonal antibodies used in testing for drugs of abuse such as antibodies to tetrahydrocannabinol, benzodiazapine, or phenobarbital. The cross-reactive substance or substances are metabolites of fenoprofen that are believed to have little structural resemblance with the analytes to be determined. In order to achieve a more specific assay, it is important that the binding of the cross-reactive antibodies to the interfering metabolites be substantially reduced or eliminated.

Samples to be assayed for the presence of the thyroid hormones triiodothyronine and tetraiodothyronine also contain thyroxine binding globulin (TBG) which binds to the triiodothyronine or tetraiodothyronine. As a result, quantitation of such analytes in untreated samples is not possible with an immunoassay because a number of molecules of the material free from complexation with TBG will vary with the TBG concentration of the sample. Consequently, it is necessary to disrupt the binding of the triiodothyronine or tetraiodothyronine with TBG prior to or simultaneous with conducting an assay without interfering with binding of the analyte with antibody used in the assay. One compound that has been utilized to displace triiodothyronine and tetraiodothyronine from TBG is 8-anilinoanthralene-1-sulfonic acid.

The use of anilinoanthralene sulfonic acid for displacing tetraiodothyronine from TBG in assays for tetraiodothyronine is described in U.S. Patent Nos. 3,928,553 and 3,911,096. European Patent Application 0,133,464 describes the use of 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid and its salts as thyroxine binding protein blocking agents for use in immunoassays and also refers to U.S. Patent No. 4,468,469 as disclosing substituted phenylacetic acids.

One aspect of the present invention concerns methods for inactivating interfering binding proteins in an immunoassay for an sbp member such as a ligand without interfering with the binding of the sbp member with its receptor, such as an antibody. Another aspect of the invention concerns methods for essentially completely disrupting complex formation between a ligand and a binding protein. The methods comprise including in a medium containing a ligand an effective amount of a water soluble compound having a structure comprised of two substituted or unsubstituted phenyl groups linked to a common atom wherein the structure may be further substituted. When the ligand has two phenyl groups linked to a common atom, the compound utilized in the method of the invention has a number of groups other than hydrogen attached to the phenyl groups and the common atom that differs from the number of such groups on the ligand by at least two. When the ligand has two phenyl groups linked to a common atom and the binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom.

Compounds useful in the method of the invention have the following formula:



wherein:

X is selected from the group consisting of O, NH, P, N, C, S, and CH₂ wherein the unsatisfied valencies of N, C, P, and S can be satisfied by single or double bonds to O, S, or C atoms which are parts of groups having molecular weights of from 14 to 200, such groups being comprised of hydrogen, carbon, nitrogen, oxygen and sulfur;

R groups are independently groups of 1 to 12 atoms other than hydrogen which may include a chain of 1-10 atoms other than hydrogen or can be taken together, or taken with X, to form a non-benzenoid ring having three to eight atoms other than hydrogen, which atoms referred to in all of the above are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, phosphorus and sulfur wherein at least one R contains a water solubility imparting moiety; and

m is a number between 0 and 5,

with the proviso that when the ligand has two phenyl groups linked to a common atom, the compound utilized in the method of the invention has a number of groups other than hydrogen attached to the phenyl groups and the atom that differs from the number of such groups on the ligand by at least two and when the ligand has two phenyl groups linked to a common atom and the binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom.

Another aspect of the invention concerns a method for reducing interference by fenoprofen and its metabolites in an analyte binding assay. The method comprises contacting the binding protein used in assaying a sample suspected of containing the analyte with an effective amount of one of the aforementioned compounds.

Another aspect of the present invention concerns a method for releasing thyroid hormones from thyroxine binding globulin in a sample to be analyzed without interfering with binding of thyroid hormones to antibodies used in the assay. Such method comprises contacting a sample to be analyzed with an effective amount of one of the compounds mentioned above.

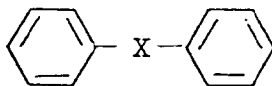
The invention further includes certain N-(3-phenoxybenzoyl)amino acid derivatives, compositions comprising such derivatives, and kits for conducting an assay.

One aspect of the present invention concerns compounds useful to prevent binding of an analyte to a first class of binding proteins in an assay so as to permit binding of the analyte to a second class of binding proteins employed as an assay reagent where the first class of binding proteins is present in the analytical sample or is a subset of the second class of binding proteins, usually antibodies, that is cross-reactive with the analyte and interfering substances. A requirement is, therefore, that the compound does not bind competitively with analyte to the antibody population required for the assay.

As mentioned above, another aspect of the present invention concerns methods for essentially completely disrupting complex formation between a ligand and a binding protein. The methods comprise including in a medium containing a ligand and a binding protein an effective amount of a water soluble compound having a structure comprised of two substituted or unsubstituted phenyl groups linked to a common atom wherein the structure may be further substituted. When the ligand has two phenyl groups linked to a common atom, the compound useful in the invention has a number of groups other than hydrogen attached to the phenyl groups and the common atom which number differs from the number of such groups on the ligand by at least two. When the ligand analyte has two phenyl groups linked to a common atom and the binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or to the common atom.

By the term "essentially completely disrupting complex formation" is meant that the compound of the invention either prevents substantial binding between the ligand and a binding protein or, where a complex is already formed between the ligand and a binding protein, the compound of the present invention acts in such a way as to render the ligand substantially free of complexation with the binding protein. The binding protein will be a subset of all the binding proteins present in the medium. In both situations usually at least 80% of the interfering binding protein will be prevented from binding the ligand, frequently 90 percent, preferably at least 99%.

By the term "substituted" is meant that a C, N, P, or S atom in the structure



is bound to a group other than hydrogen. The exact nature of such group will be evident from the description below. Conversely, by the term "unsubstituted" is meant that the atom has no substituent or is bound to hydrogen. The groups or substituents may include as part thereof alkyl, including lower alkyl of 1 to 5 carbon atoms and alkyl of 6 to 12 carbon atoms, hydroxy, -O-, -S-, COOH, SO₃H, OPO₃H₂, C=O, =O, CONH, NH₂, -NH-, SH, halo, NO₂, CN, aryl or heterocyclic groups,

Before proceeding further with a description of the present invention, a number of terms will be defined.

Ligand -- any organic compound for which a binding protein naturally exists or can be prepared. The ligand can be an analyte or a compound present in a sample containing the analyte that interferes with detection of the analyte.

Analyte--a compound or composition to be measured that is capable of binding specifically to a binding protein, usually an antigen such as a drug; a member of a specific binding pair.

The precise nature of drug analytes together with numerous examples thereof are disclosed in U.S. Patent 4,299,916 to Litman, et al., particularly columns 16 to 23, and in U.S. Patent No. 4,275,149, columns 17 and

18.

The monoepitopic ligand analytes will generally be from about 100 to 2,000 molecular weight, more usually from 125 to 1,000 molecular weight. The analytes of interest include drugs, metabolites, pesticides, pollutants, and the like. Included among analytes of interest are the thyroid hormones triiodothyronine and tetraiodothyronine. Also included among drugs of interest are the alkaloids. Among the alkaloids are morphine alkaloids, which includes morphine, codeine, heroin, dextromethorphan, their derivatives and metabolites; cocaine alkaloids, which include cocaine and benzoyl ecgonine, their derivatives and metabolites, ergot alkaloids, which include the diethylamide of lysergic acid; steroid alkaloids; imidazol alkaloids; quinazoline alkaloids, isoquinoline alkaloids; quinoline alkaloids, which include quinine and quinidine; diterpene alkaloids, their derivatives and metabolites.

The next group of drugs includes steroids, which includes the estrogens, androgens, and corticosteroids, bile acids, cardiotonic glycosides and aglycones, which includes digoxin and digoxigenin, saponins and saponinogens, their derivatives and metabolites. Also included are the steroid mimetic substances, such as diethylstilbestrol.

The next group of drugs is lactams having from 5 to 6 annular or ring members, which include the barbiturates, e.g. phenobarbital and secobarbital, diphenylhydantoin, primidone, ethosuximide, and their metabolites.

The next group of drugs is aminoalkylbenzenes, with alkyl of from 2 to 3 carbon atoms, which includes the amphetamines, catecholamines, which includes ephedrine, L-dopa, epinephrine, norepinephrine, papaverine, and their metabolites.

The next group of drugs is benzheterocyclics which include oxazepam, chlorpromazine, tegretol, imipramine, their derivatives and metabolites, the heterocyclic rings being azepines, diazepines and phenothiazines.

The next group of drugs is purines, which includes theophylline, caffeine, their metabolites and derivatives.

The next group of drugs includes those derived from marijuana, such as the cannabinoids which include cannabidiol and tetrahydrocannabinol.

The next group of drugs includes the vitamins such as A, B, e.g., B₁₂, C, D, E and K, folic acid, and thiamine.

The next group of drugs is prostaglandins, which differ by the degree and sites of hydroxylation and unsaturation.

The next group of drugs is antibiotics, which include penicillin, chloramphenicol, actinomycin, tetracycline, terramycin, the metabolites and derivatives.

The next group of drugs is the nucleosides and nucleotides, which include ATP, NAD, FMN, adenosine, guanosine, thymidine, and cytidine with their appropriate sugar and phosphate substituents.

The next group of drugs is miscellaneous individual drugs which include methadone, meprobamate, serotonin, meperidine, lidocaine, procaineamide, acetylprocaineamide, propranolol, griseofulvin, valproic acid, butyrophenones, antihistamines, anticholinergic drugs, such as atropine, their metabolites and derivatives.

The next group of drugs is the tricyclic antidepressants including derivatives of dibenzazepine, dibenzocycloheptadiene, and dibenzoxepin such as amitriptyline, nortriptyline, imipramine, desipramine, protriptylene, trimipramine, clomipramine, and doxepin.

Metabolites related to disease states include spermine, galactose, phenylpyruvic acid, and porphyrin Type

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The next group of drugs is aminoglycosides, such as gentamicin, kanamycin, tobramycin, and amikacin.

Among pesticides of interest are polyhalogenated biphenyls, phosphate esters, thiophosphates, carbamates, polyhalogenated sulfenamides, their metabolites and derivatives.

The invention has particular application to cannabinoids, methaqualone, benzodiazepines, barbiturates, phenytoin, thyronines, tricyclic antidepressants, and the like.

Member of a specific binding pair ("sbp member")--one of two different molecules having an area on the surface or in a cavity which specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as ligand and receptor (antiligand). These will usually be members of an immunological pair such as antigen-antibody, although other specific binding pairs such as biotin-avidin, hormone-hormone receptor, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like are not immunological pairs but are included in the definition.

Binding Protein--any compound or composition capable of recognizing a particular spatial and polar organization of a molecule, e.g., epitopic or determinant site. Illustrative binding proteins include naturally occurring receptors, e.g., thyroxine binding globulin, antibodies, enzymes, Fab fragments, lectins, and the like. When several related binding proteins are used such as subsets of polyclonal antibodies, the binding protein may be one such subset.

Antibody -- an immunoglobulin, or derivative or fragment thereof, having an area on the surface or in a

cavity which specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of another molecule. The antibody can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art such as, for example, immunization of a host and collection of sera or hybrid cell line technology.

5 Antibody for the analyte -- an antibody specific for an analyte.

The method of the invention has application where the ligand analyte has the formula:



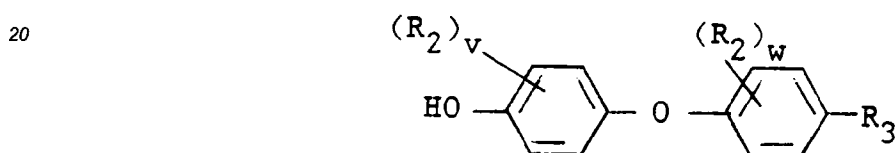
wherein:

10 A is a group having the structure E(D) where E is a substituted or unsubstituted 6-membered ring such as phenyl, pyridyl, piperidyl, pyranyl, cyclohexyl, etc.; D is a substituted or unsubstituted phenyl ring bound to E through a bond or through an atom selected from the group consisting of oxygen (O), sulfur (S), nitrogen (N), phosphorus (P) or carbon (C);

R₁ is each independently selected from the group consisting of a chain of 1-10 atoms other than hydrogen consisting of halogen, carbon, nitrogen, oxygen, and sulfur and are substituents on A connected by single or double bonds, or can be taken together to form a ring; and

15 q is 1 to 6.

In one particular aspect a ligand has the formula:



25 wherein:

R₂ in the above formula is independently selected from the group consisting of hydrogen, halogen, including bromine, chlorine, iodine, and lower alkyl of from 1 to 4 carbon atoms, including methyl, ethyl, isopropyl, and tert-butyl;

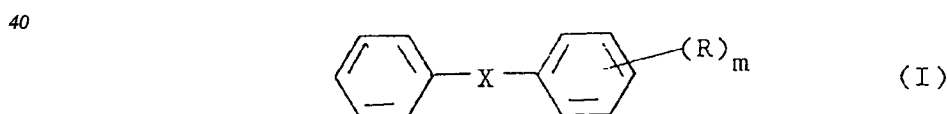
30 R₃ in the above formula is a group that consists of from one to ten atoms other than hydrogen, which atoms are independently selected from the group consisting of one or more of carbon, oxygen and nitrogen. The group may contain a carboxylic acid, ester, or amide functionality; and

v and w are independently 0 to 2.

Illustrative compounds are 3', 3'', 5'-triiodothyronine, and 3', 3'', 5', 5''-tetraiodothyronine.

35 As mentioned above, other analytes of special interest are cannabinoids, methaqualone, benzodiazepines, barbiturates, phenytoin, thyronines, and tricyclic antidepressants.

Compounds useful in the methods of the present invention are preferably water soluble and have the following formula:



45 wherein:

X is selected from the group consisting of O, NH, P, N, C, S, and CH₂ wherein the unsatisfied valencies of N, C, P and S can be satisfied by single or double bonds to O, S, or C atoms which are parts of groups having molecular weights of 14 to 200 comprised of hydrogen, carbon and nitrogen; in one embodiment both hydrogens on a carbon atom may be replaced by a double bond to O, C or N or by single bonds to one atom of a ring having three to eight atoms other than hydrogen, which atoms are independently selected from the group consisting of carbon, nitrogen, oxygen and sulfur;

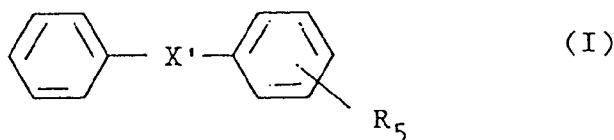
50 R groups are independently groups of 1 to 12 atoms other than hydrogen which may include a chain of 1-10 atoms other than hydrogen or can be taken together with each other or with X to form a non-benzenoid ring having five to eight atoms other than hydrogen, which atoms referred to in all of the above are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, phosphorus and sulfur wherein at least one R contains a water solubility imparting moiety; and

m is a number between 0 and 5,

with the proviso that when the ligand has two phenyl groups linked to a common atom, the compound

utilized in the method of the invention has a number of groups other than hydrogen attached to the phenyl groups and the common atom that differs from the number of such groups on the ligand by at least two and when the ligand has two phenyl groups linked to a common atom and the class of binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom.

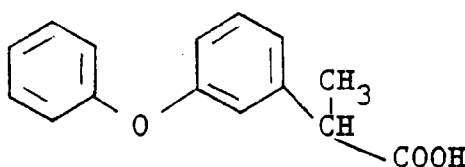
Preferred compounds for use in the methods of the present invention have the following formula:



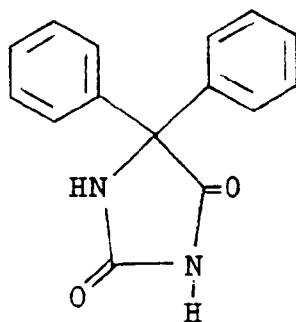
wherein:

X' in the above formula is selected from the group consisting of O, NR₄, S(O)_y, C=O, and C(R₄)₂, wherein y is a number from 0 to 2 and R₄ is independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms or wherein, alternatively, R₄ can be taken together to form a ring having five to eight atoms other than hydrogen, which atoms are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur. The ring may contain, by way of example and not limitation, -CONHCONH-, -CH₂CH₂CH₂CH(COOH)-, -OCH₂CH₂O-, -(CH₂)₄-, -(CH₂)₂NH(CH₂)₂-;

R₅ in the above formula is a group of from one to twelve atoms, preferably three to twelve atoms, other than hydrogen, which atoms are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, phosphorus and sulfur; preferably, R₅ includes a water solubility imparting moiety. A group or functionality imparting water solubility is a hydrophilic functionality which renders the compound soluble in water to an extent of at least one micromolar. Such functional group or functionality can include a sulfonate, phosphate, phosphonate, carboxylate, hydroxyl, amine, ether, amide, and the like. Illustrative R₅ groups are carboxyalkyl, sulfonoxalkyl, CONHOCH₂COOH, CO-NH (glucose), SO₂NHCH₂COOH, SO₃H, CONHCH₂CH₂SO₃H, PO₃H₂, OPO₃H₂, hydroxyl, carboxyl, ketone, and combinations thereof. For example, a preferred compound of the invention may be of the formula:



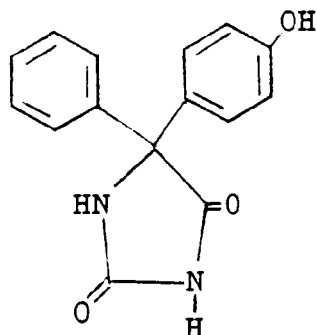
As mentioned above, when the ligand has two phenyl groups linked through an atom, the compound has a number of groups other than hydrogen attached to the phenyl groups and the atom. This number differs from the number of such groups on the ligand by at least two. For example, the analyte in an assay may be diphenylhydantoin, which has the structure:



A metabolite of diphenylhydantoin which may cross react in an assay has the structure:

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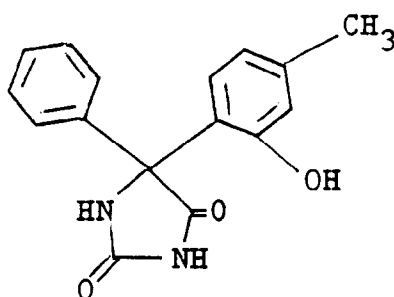


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Accordingly, compounds used in accordance with the present invention for essentially completely disrupting complex formation between the metabolite and a subset of antibodies that bind to the metabolite and to diphenylhydantoin would be compounds of formula I such as:

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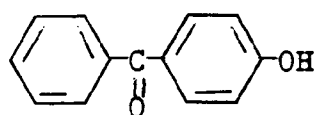
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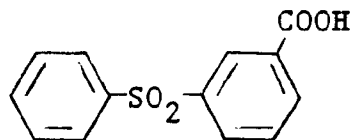
COMPOUND IA

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COMPOUND IB



COMPOUND IC

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As can be seen, compound IA has two groups, namely, $-CH_3$ and $-OH$, on a phenyl group whereas the ligand diphenylhydantoin has hydrogens in the corresponding positions. Compound IB has an oxygen on the atom joining the phenyl groups and an OH on the phenyl ring whereas diphenylhydantoin has a five-membered ring on the atom joining the phenyl groups and a hydrogen in the corresponding position on a phenyl ring. Compound IC differs from diphenylhydantoin in having a sulfur as a linking atom with two oxygens and a carboxyl group on a phenyl ring.

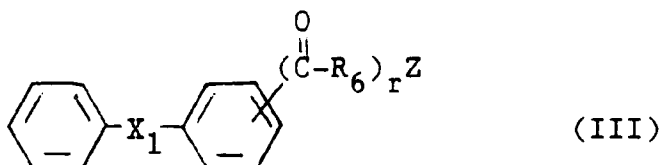
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Further, as mentioned above, when the ligand has two phenyl groups linked to a common atom and the first binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom. For example, for a thyronine analyte in serum, thyroxine binding globulin (TBG) is present and binds thyronine, thus increasing the potential for an inaccurate assay. TBG is a binding protein but is not an antibody. Therefore, a compound for inactivating TBG in an assay for a thyronine analyte might be, for example, compound IIIG or IIH (*infra*), which have only one group other than hydrogen attached to a phenyl group or the common atom.

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More preferred compounds for use in the methods of the present invention have the formula:

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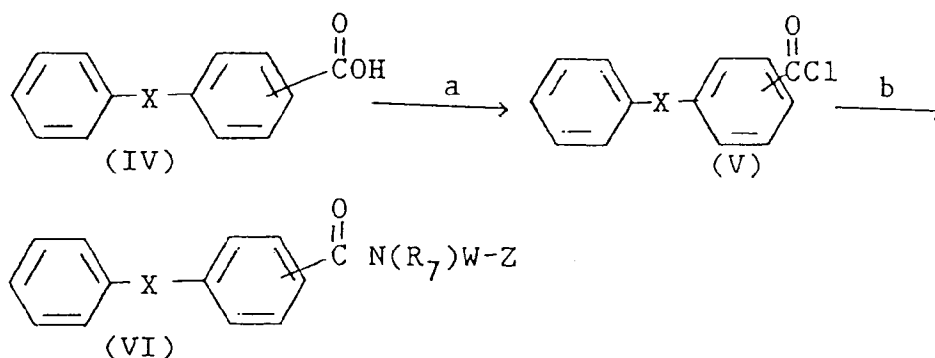
(III)

wherein:

R_6 in the above formula is $TN(R_7)W$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms. The linear or branched chain may include a non-oxocarbonyl group, (which may be one of the following groups: carboxylic acid, ester, amide, sulfonamide, sulfonic acid, halide and anhydride). R_7 in the above formula is H, or alkyl of 1 to 5 carbon atoms, for example, CH_3 or CH_2CH_3 and r is zero or 1. Z in the above formula is $COOH$, SO_3H , or PO_3H_2 ; and X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 is independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms and t is zero to 2.

Particularly preferred compounds for use in the methods of the present invention are the following: IIIA wherein r is zero, X_1 is O and Z is $COOH$; IIIB wherein r is 1, T is a bond, R_7 is CH_3 , W is CH_2 , Z is SO_3H , and X_1 is O; IIIC wherein r is 1, T is a bond, R_7 is CH_3 , W is CH_2 , Z is $COOH$, and X_1 is 0; IIID wherein r is 1, T is a bond, R_7 is H, W is CH_2 , Z is $COOH$, and X_1 is 0; IIIE wherein r is 1, T is a bond, R_7 is H, W is $CH(CH_3)$, Z is $COOH$, and X_1 is 0; IIIF wherein r is 1, T is a bond, R_7 is H, W is OCH_2 , Z is $COOH$, and X_1 is 0; IIIG wherein r is 1, T is a bond, R_7 is H, W is CH_2CH_2 , Z is SO_3H , and X_1 is 0; IIH wherein r is 1, T is a bond, R_7 is H, W is CH_2CH_2 , Z is $COOH$, and X_1 is 0; IIJ wherein r is 1, T is a bond, R_7 is H, W is CH_2CH_2 , Z is SO_3H , and X_1 is CH_2 ; and IIK wherein r is 1, T is a bond, R_7 is CH_3 , W is CH_2CH_2 , Z is SO_3H , and X_1 is 0.

The compounds useful in the methods of the present invention can be prepared according to the following general procedure:



wherein X, R_7 , W, and Z are as defined above; step (a) involves formation of an acid chloride by treatment with, for example, thionyl chloride, oxalylchloride, or phosphorous oxychloride, under conventional conditions; and step (b) involves treatment of an acid chloride with $H_2N(R_7)W-Z$ under conditions for nitrogen displacement of chloride to form an amide linkage, such as methanol and triethyl amine, or the like.

Compound IV where X is O is commercially available. Compound IV where X is $C=O$ may be prepared by a procedure similar to that described in J. Org. Chem. (1985) 50(16): 2093-2904

Compound IV where X is S may be prepared by a procedure similar to that described in Ger. Offen. DE 2619489 (1976).

Compound IV where X is SO_2 may be prepared by a procedure similar to that described in Ger. Offen. DE 2252014 (1973)

Compound IV where X is NH is discussed in Org. Mass Spectrom. (1984) 19(9): 438-441

In carrying out the methods of the invention an aqueous medium will normally be employed. Other polar solvents may also be employed, usually oxygenated organic solvents of from 1-6, more usually from 1-4 carbon atoms, including alcohols, ethers and the like. Usually these cosolvents will be present in less than about 40 weight percent, more usually in less than about 20 weight percent.

The pH for the medium will usually be in the range of about 4-11, more usually in the range of about 5-10, and preferably in the range of about 5.4-9.5. The pH is chosen so as to maintain a significant level of disruption of binding between a ligand and a binding protein while optimizing binding between an analyte and its complementary binding protein. In some instances, a compromise will be made between these two considerations. Various buffers may be used to achieve the desired pH and maintain the pH during the determination. Illustrative buffers include borate, phosphate, carbonate, Tris, barbital and the like. The particular buffer employed is not critical to this invention but in individual assays, one buffer may be preferred over another.

Moderate temperatures are normally employed for carrying out the methods and usually constant temperatures during the period of the methods. The temperatures for the determination will generally range from about $10^\circ\text{--}50^\circ\text{C}$., more usually from about $15^\circ\text{--}40^\circ\text{C}$.

An effective amount of a compound used in accordance with the invention to essentially completely disrupt

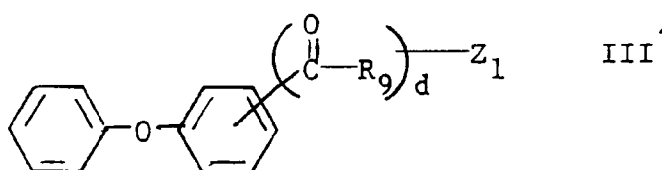
complex formation between a ligand and a binding protein is at least 1 μM and will generally vary from about 10 μM to 10 mM, more usually from about 100 to 1000 μM

Where the present method is used in conjunction with an assay, considerations such as whether the assay is qualitative, semi-quantitative or quantitative, the particular detection technique, and the concentration of the analyte will normally determine the concentration of the other reagents. While the concentrations of the various reagents will generally be determined by the concentration range of interest of the analyte, the final concentration of each of the reagents will normally be determined empirically to optimize the sensitivity of the assay over the range of interest. The total binding sites of the members of the specific binding pair which are complementary to the analyte will be not less than about 0.1 times the minimum concentration of interest based on binding sites of the analyte and not more than about 10,000 times the maximum concentration of interest based on analyte binding sites, usually about 0.1-1000 times, more usually about 0.3-10 times, the maximum concentration of interest. For ligand analyte, where labelled ligand is employed, the concentration range of the labelled ligand based on equivalents will generally be not less than about 10^{-6} , more usually not less than 10^{-2} , times the minimum concentration of interest and not greater than 100, usually not greater than 10, times the maximum concentration of interest.

The order of addition of the various reagents may also vary and is dependent on what type of assay is utilized wherein many of the same considerations mentioned above apply.

The present method has application both to heterogeneous and homogeneous assays. Exemplary heterogeneous assays are found in U.S. Patent Nos. 4,256,834 and 4,261,968. Homogeneous immunoassays are exemplified by immunofluorescence methods such as those disclosed in U.S. Patent No. 3,993,345, enzyme channeling techniques such as those disclosed in U.S. Patent No. 4,233,402, and other enzyme immunoassays as discussed in Maggio, *supra*, and in U.S. Patent No. 3,817,837. The assay can be competitive or direct and can involve compounds of the invention that are either labeled ligand or labeled receptor.

A particular embodiment of the invention concerns a method for reducing interference by fenoprofen and its metabolites in an analyte binding assay. The method comprises contacting the antibody used in assaying a sample suspected of containing the analyte with an effective amount of a compound of the formula:



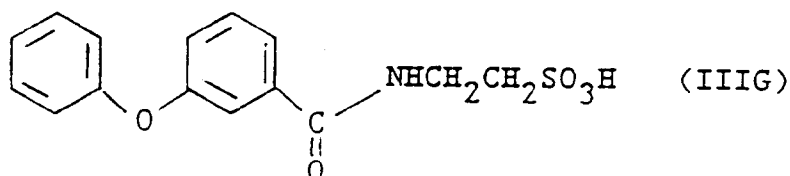
wherein:

R_9 is $T_1N(R_{10})W_1$ wherein T_1 and W_1 are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms and R_{10} is H or lower alkyl of from 1 to 5 carbon atoms, d is 0 or 1, and

Z_1 is COOH , SO_3H , or PO_3H_2 .

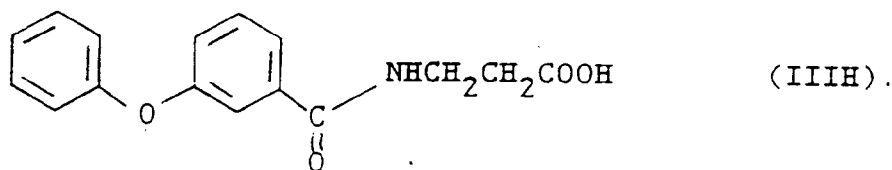
Another embodiment of a method in accordance with the present invention is a method for releasing thyroid hormones such as triiodothyronine and tetraiodothyronine from thyroxine binding globulin in a sample to be analyzed. The method comprises contacting said sample with an effective amount of compound III.

A particular aspect of the present invention includes the use of a compound of the formula:



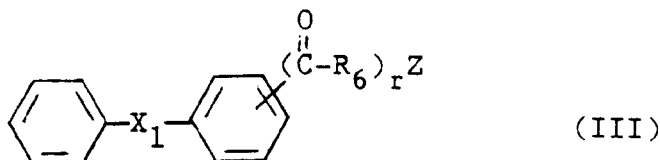
and

in an in-vitro immunoassay; as well as a compound of the formula:



Compound (IIIG) is described in Pestic. Sci 1981 12, 385-398.

Also included within the scope of the invention are compositions comprising a sample to be analyzed and a compound of the invention such as those of formula (III):



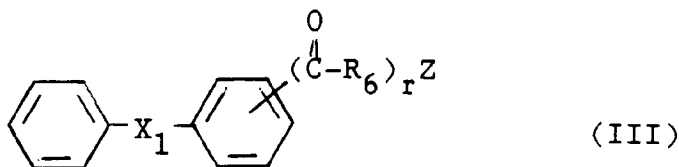
wherein:

20 R_6 in the above formula is $TN(R_7)W$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms. The linear or branched chain may include a non-oxocarbonyl group, (which may be one of the following groups: carboxylic acid, ester, amide, sulfonamide, sulfonic acid, halide and anhydride). R_7 in the above formula is H, or alkyl of 1 to 5 carbon atoms, for example, CH_3 or CH_2CH_3 and r is zero or 1.

25 Z in the above formula is $COOH$, SO_3H , or PO_3H_2 ; and X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 is independently selected from the group consisting of hydrogen and lower alkyl and t is zero to 2.

Particularly preferred compositions are those comprising a sample to be analyzed and a compound of the invention such as IIIG or the compound IIIH, as defined above.

30 As a matter of convenience, the reagents employed in the present invention can be provided in a kit in packaged combination in predetermined amounts. The reagents can be separately contained or two or more reagents can be combined in a single container depending on their stability and cross-reactivity. For use in assaying for an analyte in a sample, the reagents may include a compound of the invention as disclosed above, and, where appropriate, conjugates of signal generating compounds and specific binding pair members or other reaction partners to provide the detectable signal. In addition, other additives such as ancillary reagents may be included. The relative amounts of the various reagents may be varied widely, to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of an assay. The reagents can be provided as dry powders, usually lyophilized, including excipients, which on dissolution will provide for a reagent solution having the appropriate concentrations for performing an assay. Preferably, kits of the invention will contain the compounds of formula (III):



wherein:

50 R_6 in the above formula is $TN(R_7)W$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms. The linear or branched chain may include a non-oxocarbonyl group, (which may be one of the following groups: carboxylic acid, ester, amide, sulfonamide, sulfonic acid, halide and anhydride). R_7 in the above formula is H, or alkyl of 1 to 5 carbon atoms, for example, CH_3 or CH_2CH_3 and r is zero or 1.

55 Z in the above formula is $COOH$, SO_3H , or PO_3H_2 ; and X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 is independently selected from the group consisting of hydrogen and lower alkyl and t is zero to 2.

EXAMPLES

The invention is further demonstrated by the following illustrative examples. Parts and percentages are by weight to volume unless otherwise indicated.

EXAMPLE 1Preparation of m-(phenoxy)benzoylchloride

In a well ventilated hood, a mixture of m-phenoxy benzoic acid (200 g, 0.933 mole) (Aldrich) and thionyl chloride (105 mL, 1.44 moles) was placed in a one liter, round-bottomed flask. The flask was fitted with a magnetic stirring bar (or mechanical stirring bar for larger quantity preparation) and a reflux condenser with calcium chloride drying tube, which was attached to an exit tube, for evolved hydrogen chloride and sulfur dioxide, leading to a gas absorption trap. The mixture was heated on a heating mantle cautiously at first until a brownish-green solution was obtained (about 1 1/2 hours). The resulting solution was then heated gently for an additional 1 1/2 hours. The resulting brown solution was then cooled, and excessive thionyl chloride was distilled off at 40°C. To the resulting oil was added toluene (75 mL), and the solution was evaporated to remove a small amount of thionyl chloride. Addition and evaporation of toluene (75 mL) was repeated once more. The resulting acid chloride was then dissolved in 100 mL of tetrahydrofuran (THF dried over molecular sieves 3A), and ready to use freshly for reaction.

EXAMPLE 2Preparation of N-(m-phenoxybenzoyl) tauric acid (PBTA)

A solution of taurine (116 g, 0.927 mole) in a mixture of solution containing tetrahydrofuran (300 mL), 40% sodium hydroxide (43.5 mL) and water (940 mL) was placed in a three-necked, round-bottomed flask (3 liters). The reaction flask was fitted to a pH meter and equipped with two addition funnels. The m-phenoxybenzoyl chloride (prepared in Example 1) in THF solution was added slowly, through the addition funnel protected from moisture using calcium chloride drying tube, to the taurine solution (pH adjusted to 8.9 before reaction) at room temperature. Simultaneously, a solution of 40% sodium hydroxide (total of 155 mL) was added slowly through the second addition funnel to adjust the pH of the reaction between 8.5 and 9. The addition took 5 to 7 hours. The resulting yellow solution was allowed to stand at room temperature overnight. Complete reaction was observed on thin layer chromatography (tlc).

The product was then acidified using concentrated HCl (about 4.5 mL) to pH 2.5, and the aqueous solution was extracted using ether (20 x 100 mL) until the complete removal of starting material (monitored by TLC, silica gel plate, 0.1:2:8:acetic acid:CH₃OH:CH₂Cl₂). To the resulting solution (about 1.4 liters) was slowly stirred in powdered sodium chloride (50 grams) until sodium chloride dissolved completely, and the solution was mixed with 1 liter of 35% sodium chloride solution. The crude product (about 360 g) thus obtained was recrystallized in 1.4 liters of 1:1/MeOH:9% NaCl. The resulting white crystals were collected by suction and dried overnight. The suctioned dried crystals were transferred to a vacuum desiccator and dried at room temperature for 48 hours or until constant weight. The product (267 g, one spot on TLC, R_f 0.68, butanol:methanol:benzene:water/2:1.25:1:1, silica gel plate) showed NMR (D₂O) peaks at 3.04 ppm (t, 2H), 3.63 ppm (t, 2H), 6.89 ppm - 7.39 ppm (m, 9H) and UV λ max. at 278 nm.

EXAMPLE 3

The purpose of this experiment was to study the effects of PBTA as a dissociating agent for measuring thyroxine (T4) in a sample population. An Emit® (Syva Company, Palo Alto, CA) ACA® T4 assay (E. 1. DuPont de Nemours Company, Wilmington, Delaware) was used as a model system for this experiment.

Materials

The antibody reagent contains antibodies from sheep immunized with a protein-T4 conjugate, stabilizers and preservatives in a 0.055 M Tris solution (pH 6.0). Enzyme reagent contains a T4 analog chemically coupled to glucose-6-phosphate dehydrogenase, stabilizers and preservatives in a 0.055 M Tris solution (pH 7.0). PBTA was formulated in a 0.1 M Tris buffer (pH 9.0) with preservatives at 5, 10 and 20 mg/mL concentrations. For purposes of comparison, 8-anilino-naphthalenesulfonic acid (ANS, a recognized T4 dissociating agent) was for-

mulated in a 0.1 M Tris buffer at 3 mg/mL.

The ACA T4 assay pack configuration was as follows:

Dimple 1 - Serum treatment

Dimple 2 - Buffer tab

5 Dimple 3 - G6P/NAD tab

Dimple 4 - Antibody reagent

Dimple 5 - Empty

Dimple 6 - Enzyme reagent

Dimple 7 - Empty

10 Each pack was self contained for one sample determination. The volume of antibody, enzyme and serum treatment (PBTA or ANS) was 60 µL each for all tests.

Protocol

15 For each pack run, ACA instrument injected 200 µL of calibrator or sample and 4800 µL of water into the pack. The pack was then preheated to 37°C during a 100 second incubation. The first four dimples were then opened and mixed with the diluted sample. After a four minute incubation, the last three dimples were opened and mixed in with the sample. Absorbance readings were started 30 seconds later for a kinetic rate determination. Optical measurements were taken for 17 seconds. All operations of the ACA were handled automatically and were set by the manufacturer.

20

Results

25 The rates and quantitations are summarized in Table 1. Reference data on the samples were obtained independently by a different methodology (Clinical Assays Total T₄ Radioimmunoassay (RIA)) to assess any differences between the serum treatments. All quantitation data was generated using a log-logit equation to generate a standard curve from the calibrator rates and assigned concentrations.

Scattergrams and linear regression statistics were generated to fully assess the differences, if any, between the serum treatment reagents. The results are summarized in Table 1.

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Table 1

Additive	Reagent ¹ conc. (mg/mL)	Final ² conc. (mM/L)	Assay range ³	%Modulation ⁴	Corr Coeff.	SEE ⁵
PBTA	—	—	260	37.5	.950	1.142
PBTA	5	0.186	300	35.5	.958	1.170
PBTA	10	0.374	360	38.8	.977	0.759
PBTA	20	0.748	354	36.7	.982	0.694
ANS	3	0.120	342	38.3	.968	0.823

1. Reagent conc. = Additive concentration in
the serum treatment reagent

2. Final conc. = Additive concentration in
the final reaction mixture

3. Assay range = Rate of #5 calibrator minus
rate of #1 calibrators

4. % Modulation = $\frac{(\#5 \text{ cal rate minus } \#1 \text{ cal rate}) \times 100}{\#5 \text{ cal rate}}$

5. SEE = Standard error of the estimate.

cal = calibrator

Discussion.

It is clear from the data in the table that the serum treatments have an effect on the assay. The rates from the standard curves increase along with the increasing concentration of PBTA. This indicates that PBTA is affecting the ability of serum proteins to bind to T4. This effect can also be seen on the scattergrams. The data points on the scattergrams fit in a tighter distribution about the linear regression line with higher PBTA concentrations. Notice that above the threshold concentration of 5mg/mL PBTA the Standard Error of the Estimate (SEE) drops indicating that sample to sample differences on binding proteins is being overcome. ANS is widely used as a T4 dissociating agent and when used in this assay configuration produced comparable data as evidenced by the similarity of SEE and correlation statistics.

EXAMPLE 4

The purpose of this experiment was to study the effects of PBTA on reducing the interference of derivatives of fenopropfen in an EMIT® assay for benzodiazepines in urine. The fenopropfen derivatives are found in urine of individuals taking fenopropfen.

The antibody reagent contained antibodies from sheep immunized with a protein-diazepam conjugate, stabilizers and preservatives in a 55 mM Tris solution, pH 5.2, and glucose-6-phosphate (G6P)(66 mM) and nicotinic adenine dinucleotide (NAD⁺)(40 mM)(EMIT® benzodiazepine assay, Reagent A, Syva Company) with PBTA at 0.5 mg/ml. The enzyme reagent contained a diazepam analogue chemically coupled to glucose-6-

phosphate dehydrogenase (G6PDH), stabilizers and preservatives in a 55 mM Tris solution (pH 8.0) (EMIT® benzodiazepine assay, Reagent B, Syva Company). The assay buffer solution contained 0.5% NaCl, surfactant and preservatives in 55 mM Tris (pH 8.0). For comparison, the reagents were compared to reagents prepared and optimized without PBTA.

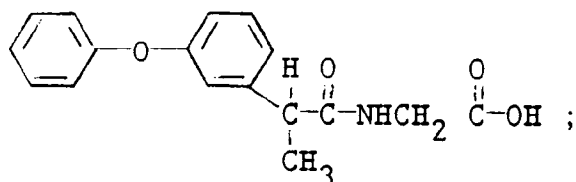
For each test, 50 µL sample, 50 µL antibody reagent, 50 µL enzyme reagent, and 750 µL assay buffer were combined and aspirated into a Gilford Stasar III flow cell at 30°C. After a delay of 15 seconds, absorbance measurements were made for 30 seconds (two per second) at a wavelength of 340 nm. A rate measurement was made by calculating the linear regression of the absorbance measurements for the 30 seconds and multiplying the subsequent rate by 2.667.

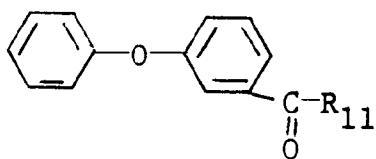
The rates for benzodiazepine calibrators using reagents with PBTA (X02) and without PBTA (W01) are shown in Table II. Rates from samples from individuals taking fenopropfen are shown relative to the value of the 300 ng/mL calibrator (+/- rate). All samples were confirmed to be negative for benzodiazepines by gas chromatography/mass spectrometry (GC/MS). The data show that the assay system containing PBTA reduced the interference of derivatives of fenopropfen in the urine sample.

TABLE II

REAGENTS	X02	W01
CALIBRATOR		
NEGATIVE	359	329
300 ng/mL	445	416
500 ng/mL	466	448
1000 ng/mL	500	502
SAMPLES		
F102R	-20	+6
F019R	-44	-9
F017R	-58	-16
F088R	-45	+4
F103Z	-48	-1
F021R	-46	-1
F104Z	-59	-6
F-BQ	-46	+7

Derivatives prepared below were as follows:





(1) glycylyl fenoprofen

(2) $R_{11} = \text{NHCH}_2\text{CH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{OH}$ β -alanine

(3) $R_{11} = \text{NHCH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{OH}$ glycine

(4) $R_{11} = \text{N}(\text{CH}_3)\text{CH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{OH}$ sarcosine

(5) $R_{11} = \text{NHCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{H}$

(6) $R_{11} = \text{NH}_2\text{OCH}_2\text{CO}_2\text{H}$

(7) $R_{11} = \text{NHCH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{NHCH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{NHCH}_2\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{OH}$

EXAMPLE 5

Preparation of N-hydroxysuccinimic ester of m-phenoxybenzoic acid

To a solution of m-phenoxybenzoic acid (2.0 g, 9.3 mmoles) in tetrahydrofuran (25 ml, freshly distilled) was added 1,3-dicyclohexylcarbodiimide (3.2 g, 15.5 mmoles) and N-hydroxysuccinimide (1.2 g, 10 mmoles). The resulting mixture was allowed to stir at room temperature under nitrogen for 18 hours, and the formation of N-hydroxysuccinimic ester of m-phenoxybenzoic acid was observed. The resulting N-hydroxysuccinimic ester was then used freshly for reaction.

EXAMPLE 6

Preparation of m-phenoxybenzoylamidopropanesulfonic acid (5)

To a mixture of 3-amino-1-propanesulfonic acid (488 mg, 3.5 mmoles), triethylamine (354 mg, 3.5 mmoles) and THF (2 ml) was added a solution of N-hydroxysuccinimic ester of m-phenoxybenzoic acid (from 500 mg of m-phenoxybenzoic acid in 6.5 ml of tetrahydrofuran described in Example 5). The resulting reaction mixture was then allowed to stir at room temperature. After 18 hours, the reaction product was acidified to pH3 and then extracted using dichloromethane to remove starting material. The resulting aqueous solution was then evaporated to dryness and to the residue was added methanol and the suspension was filtered. The filtrate thus obtained was then chromatographed on silica gel plate (20% MeOH/CH₂Cl₂) and the product was eluted using the same solvent to yield 100 mg of m-phenoxybenzoylamidopropanesulfonic acid.

EXAMPLE 7

Preparation of m-phenoxybenzoylamido-oxy-acetic acid (6)

The preparation of m-phenoxybenzoylamido-oxy-acetic acid (6) was carried out using a procedure similar to that for m-phenoxybenzoylamidopropanesulfonic acid described in Examples 5 and 6.

EXAMPLE 8

Preparation of m-phenoxybenzoyl glycylyl glycylyl glycine (7)

The preparation of m-phenoxybenzoyl glycylyl glycylyl glycine (7) was carried out using a procedure similar to that for m-phenoxybenzoylamidopropanesulfonic acid described in Examples 5 and 6.

EXAMPLE 9**Preparation of m-phenoxybenzoyl β -alanine (2)**

5 The preparation of m-phenoxybenzoyl β -alanine (2) was carried out using a procedure similar to that for N-(m-phenoxybenzoyl)tauric acid (PBTA) described in Example 2.

EXAMPLE 1010 **Preparation of m-phenoxybenzoyl glycine (3)**

The preparation of m-phenoxybenzoyl glycine (3) was carried out using a procedure similar to that for N-(m-phenoxybenzoyl)tauric acid (PBTA) described in Example 2.

15 **EXAMPLE 11****Preparation of m-phenoxybenzoyl sarcosine (4)**

20 The preparation of m-phenoxybenzoyl sarcosine (4) was carried out using a procedure similar to that for N-(m-phenoxybenzoyl)tauric acid (PBTA) described in Example 2.

EXAMPLE 12**Preparation of glycyI fenoprofen (1)**

25 A mixture of fenoprofen (130 mg, 0.53 mmole), 1,3-dicyclohexylcarbodiimide (120 mg, 0.58 mmole), N-hydroxysuccinimide (67 mg, 0.58 mmole) in tetrahydrofuran (3 ml, freshly distilled) was allowed to stir at room temperature overnight. The resulting N-hydroxysuccinimide ester of fenoprofen was then added into a solution of glycine (450 mg, 6 mmoles) in sodium bicarbonate solution (0.05 M, pH 8.6, 3 ml) with the adjustment of pH at 8.6. The resulting product was then acidified to pH 3 and extracted with ethyl acetate. The organic solvent was evaporated and the product was chromatographed on silica gel plates (butanol:methanol:benzene: water/2:1.25:1:1) and eluted using the same solvents. The solvent was then evaporated and the residue was added 10% MeOH/CH₂Cl₂ and filtered. The filtrate was evaporated to dryness to yield glycyI fenoprofen (1).

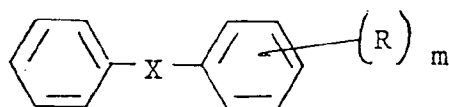
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Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

40 1. A method for inactivating interfering binding proteins in an assay for a member of a specific binding pair (sbp), which comprises including in an assay medium containing a sample suspected of containing said sbp member a water soluble compound of formula.

45



wherein:

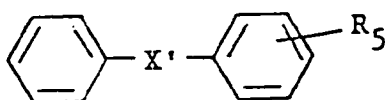
50 X is selected from the group consisting of O, NH, P, N, C, S, and CH₂ wherein the unsatisfied valencies of N, C, P and S can be satisfied by single or double bonds to O, S, or C atoms which comprise groups of molecular weight from 14 to 200, said groups having atoms independently selected from the group consisting of hydrogen, carbon, nitrogen, oxygen and sulfur;

55 R groups are independently groups of 1 to 12 atoms other than hydrogen which may include a chain of 1-10 atoms other than hydrogen or can be taken together with each other or with X to form a non-benzoid ring having five to eight atoms other than hydrogen, which atoms referred to in all of the above are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, phosphorus and sulfur wherein at least one R contains a water solubility imparting moiety; and m is a number between

0 and 5, with the proviso that, when said sbp member or its sbp partner has two phenyl groups linked through a common atom, said compound has a number of groups other than hydrogen attached to said phenyl groups and said atom that differs by at least two from the number of such nonhydrogen groups on said sbp member and when said sbp member or its sbp partner has two phenyl groups linked to a common atom and the binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom.

2. The method of Claim 1 wherein said binding protein is an antibody.

3. A method for blocking the binding by interfering binding proteins in an immunoassay for a ligand, which comprises including in a medium suspected of containing a ligand and said complementary binding protein, a water soluble compound of the following formula:



wherein:

X' is selected from the group consisting of O, NR₄, s(O)_y, C=O, and C(R₄)₂ wherein R₄ is independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms or wherein R₄ can be taken together to form a ring having five to eight atoms other than hydrogen, which atoms are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur and y is a number from 0 to 2 and,

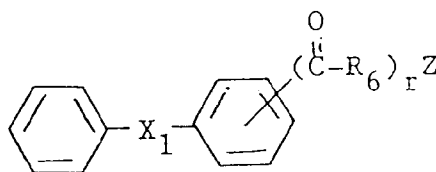
R₅ are independently selected from groups comprised of from one to twelve atoms other than hydrogen, which atoms are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, and sulfur, said group including a water solubility imparting moiety,

with the proviso that, when said ligand has two phenyl groups linked through a chain that is one atom in length, said compound has a number of groups other than hydrogen attached to said phenyl groups and said atom that differs from the number of such groups on said ligand by at least two and, when the ligand has two phenyl groups linked to a common atom and the binding protein is not an antibody, said compound has only one group other than hydrogen attached to a phenyl group or the common atom.

4. The method of Claim 3 wherein said binding protein is an antibody.

5. The method of Claim 3 wherein said binding protein is thyroxine binding globulin.

6. The method of Claim 3 wherein said compound is of the formula:



wherein

R₆ is TN(R₇)W wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R₇ is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1;

Z is COOH, SO₃H, or PO₃H₂; and

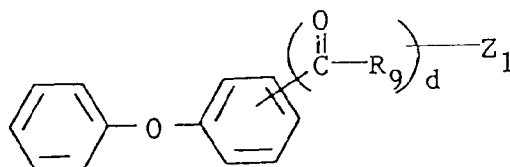
X₁ is O, NR₈, C=O, S(O)_t, C(R₈)₂ wherein R₈ are independently selected from the group consisting of hydrogen and lower alkyl and t is 0 to 2.

7. The method of Claim 6 wherein r is zero, X₁ is O and Z is COOH; or r is 1, T is a bond, R₇ is CH₃, W is CH₂, Z is SO₃H, and X₁ is O; or r is 1, T is a bond, R₇ is CH₃, W is CH₂, Z is COOH, and X₁ is O; or r is 1, T is a bond, R₇ is H, W is CH₂, Z is COOH, and X₁ is O; or r is 1, T is a bond, R₇ is H, W is CH(CH₃), Z is COOH, and X₁ is O; or r is 1, T is a bond, R₇ is H, W is CH₂CH₂, Z is SO₃H, and X₁ is CH₂; or r is 1, T is a bond, R₇ is CH₃, W is CH₂CH₂, Z is SO₃H, and X₁ is O; or r is 1, T is a bond, R₇ is H, W is OCH₂, Z is

COOH, and X_1 is 0.

8. The method of Claim 3 wherein said ligand is a thyroid hormone, phenobarbital, diphenylhydantoin, tetrahydrocannabinol, a benzodiazepine, or metabolites thereof.

9. A method for reducing interference by fenopropfen and its metabolites in an analyte binding assay which comprises contacting the binding protein used in assaying a sample suspected of containing said analyte with an effective amount of a compound of the formula:



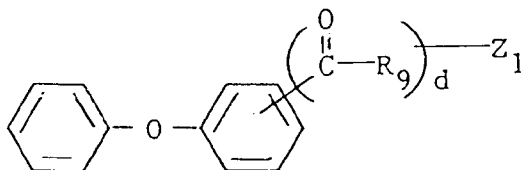
wherein

R_9 is $T_1N(R_{10})W_1$ wherein T_1 and W_1 are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms and R_{10} is H or lower alkyl of from 1 to 5 carbon atoms, d is 0 or 1, and

Z_1 is COOH, SO_3H , or PO_3H_2 .

10. The method of Claim 9 wherein d is 1, T_1 is a bond, R_{10} is H, W_1 is CH_2CH_2 and Z_1 is SO_3H .

11. A method for releasing thyroid hormones from thyroxine binding globulin in a sample to be analyzed, which comprises contacting said sample with an effective amount of a compound of the formula:



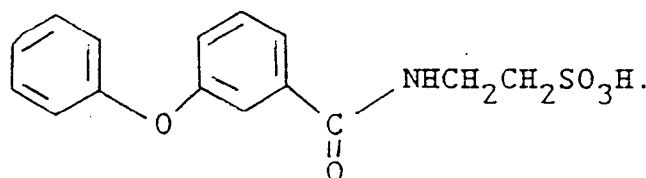
wherein

R_9 is $T_1N(R_7)W_1$ wherein T_1 and W_1 are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms and R_7 is H or lower alkyl of from 1 to 5 carbon atoms, d is 0 or 1, and

Z_1 is COOH, SO_3H , or PO_3H_2 .

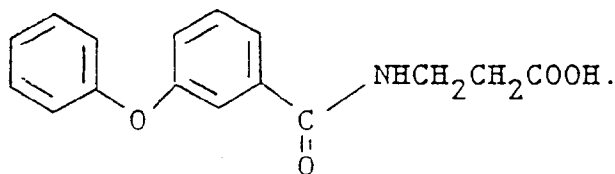
12. The method of Claim 11 wherein d is 1, T_1 is a bond, R_{10} is H, W_1 is CH_2CH_2 , and Z_1 is SO_3H .

13. The use of a compound of the formula:

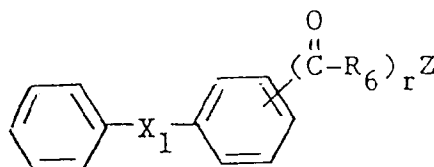


in an in-vitro immunoassay.

14. A compound of the formula:



15. A composition comprising a sample to be analyzed and the compound of formula:



wherein

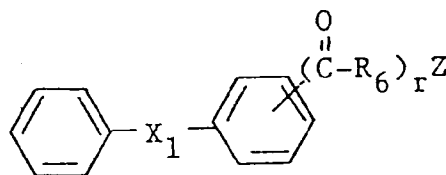
R_6 is $TN(R_7)W$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R_7 is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1;

Z is $COOH$, SO_3H , or PO_3H_2 ; and

X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 are independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms and t is 0 to 2.

16. The composition of Claim 15 comprising a sample to be analyzed and the compound of Claims 14 or 15.

17. A kit for conducting an assay comprising in packaged combination (a) the compound of formula:



wherein

R_6 is $TN(R_7)W$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R_7 is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1;

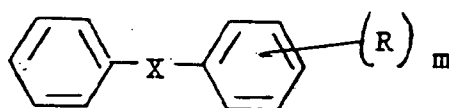
Z is $COOH$, SO_3H , or PO_3H_2 ; and

X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 are independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms and t is 0 to 2, and (b) reagents for conducting said assay.

18. The kit of Claim 17 for conducting an assay comprising in packaged combination (a) the compound of Claim 14 or 15 and (b) reagents for conducting said assay.

Claims for the following Contracting State : ES

1. A method for inactivating interfering binding proteins in an assay for a member of a specific binding pair (sbp), which comprises including in an assay medium containing a sample suspected of containing said sbp member a water soluble compound of formula.



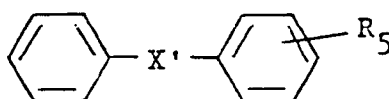
wherein:

X is selected from the group consisting of O, NH, P, N, C, S, and CH_2 wherein the unsatisfied va-

lencies of N, C, P and S can be satisfied by single or double bonds to O, S, or C atoms which comprise groups of molecular weight from 14 to 200, said groups having atoms independently selected from the group consisting of hydrogen, carbon, nitrogen, oxygen and sulfur;

R groups are independently groups of 1 to 12 atoms other than hydrogen which may include a chain of 1-10 atoms other than hydrogen or can be taken together with each other or with X to form a non-benzenoid ring having five to eight atoms other than hydrogen, which atoms referred to in all of the above are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, phosphorus and sulfur wherein at least one R contains a water solubility imparting moiety; and m is a number between 0 and 5, with the proviso that, when said sbp member or its sbp partner has two phenyl groups linked through a common atom, said compound has a number of groups other than hydrogen attached to said phenyl groups and said atom that differs by at least two from the number of such nonhydrogen groups on said sbp member and when said sbp member or its sbp partner has two phenyl groups linked to a common atom and the binding protein is not an antibody, the compound has only one group other than hydrogen attached to a phenyl group or the common atom.

2. The method of Claim 1 wherein said binding protein is an antibody.
3. A method for blocking the binding by interfering binding proteins in an immunoassay for a ligand, which comprises including in a medium suspected of containing a ligand and said complementary binding protein, a water soluble compound of the following formula:



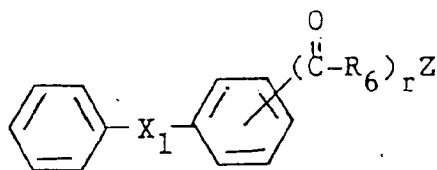
wherein:

X' is selected from the group consisting of O, NR₄, S(O)_y, C=O, and C(R₄)₂ wherein R₄ is independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms or wherein R₄ can be taken together to form a ring having five to eight atoms other than hydrogen, which atoms are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur and y is a number from 0 to 2 and,

R₅ are independently selected from groups comprised of from one to twelve atoms other than hydrogen, which atoms are independently selected from the group consisting of halogen, carbon, nitrogen, oxygen, and sulfur, said group including a water solubility imparting moiety,

with the proviso that, when said ligand has two phenyl groups linked through a chain that is one atom in length, said compound has a number of groups other than hydrogen attached to said phenyl groups and said atom that differs from the number of such groups on said ligand by at least two and, when the ligand has two phenyl groups linked to a common atom and the binding protein is not an antibody, said compound has only one group other than hydrogen attached to a phenyl group or the common atom.

4. The method of Claim 3 wherein said binding protein is an antibody.
5. The method of Claim 3 wherein said binding protein is thyroxine binding globulin.
6. The method of Claim 3 wherein said compound is of the formula:

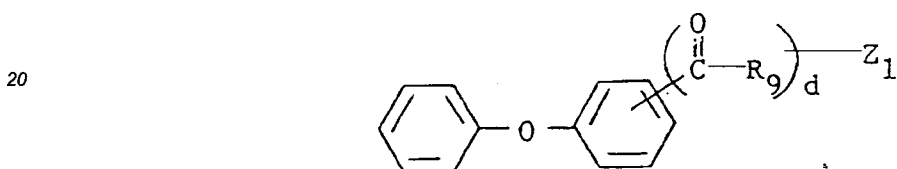


wherein

R₆ is TN(R₇)W wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R₇ is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1; Z is COOH, SO₃H, or PO₃H₂; and

X_1 is O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ wherein R_8 are independently selected from the group consisting of hydrogen and lower alkyl and t is 0 to 2.

7. The method of Claim 6 wherein r is zero, X_1 is 0 and Z is $COOH$; or r is 1, T is a bond, R_7 is CH_3 , W is CH_2 , Z is SO_3H , and X_1 is 0; or r is 1, T is a bond, R_7 is CH_3 , W is CH_2 , Z is $COOH$, and X_1 is 0; or r is 1, T is a bond, R_7 is H , W is CH_2 , Z is $COOH$, and X_1 is 0; or r is 1, T is a bond, R_7 is H , W is $CH(CH_3)$, Z is $COOH$, and X_1 is 0; or r is 1, T is a bond, R_7 is H , W is CH_2CH_2 , Z is SO_3H , and X_1 is CH_2 ; or r is 1, T is a bond, R_7 is CH_3 , W is CH_2CH_2 , Z is SO_3H , and X_1 is 0; or r is 1, T is a bond, R_7 is H , W is OCH_2 , Z is $COOH$, and X_1 is 0.
8. The method of Claim 3 wherein said ligand is a thyroid hormone, phenobarbital, diphenylhydantoin, tetrahydrocannabinol, a benzodiazepine, or metabolites thereof.
9. A method for reducing interference by fenopropfen and its metabolites in an analyte binding assay which comprises contacting the binding protein used in assaying a sample suspected of containing said analyte with an effective amount of a compound of the formula:

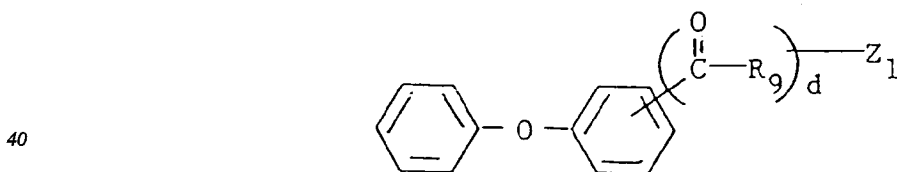


wherein

R_9 is $T_1N(R_{10})W_1$ wherein T_1 and W_1 are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms and R_{10} is H or lower alkyl of from 1 to 5 carbon atoms, d is 0 or 1, and

Z_1 is $COOH$, SO_3H , or PO_3H_2 .

10. The method of Claim 9 wherein d is 1, T_1 is a bond, R_{10} is H, W_1 is CH_2CH_2 and Z_1 is SO_3H .
11. A method for releasing thyroid hormones from thyroxine binding globulin in a sample to be analyzed, which comprises contacting said sample with an effective amount of a compound of the formula:

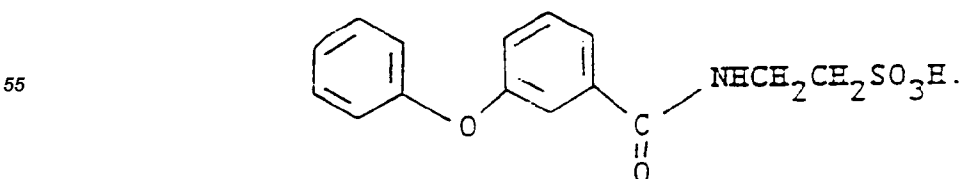


wherein

R_9 is $T_1N(R_7)W_1$ wherein T_1 and W_1 are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms and R_7 is H or lower alkyl of from 1 to 5 carbon atoms, d is 0 or 1, and

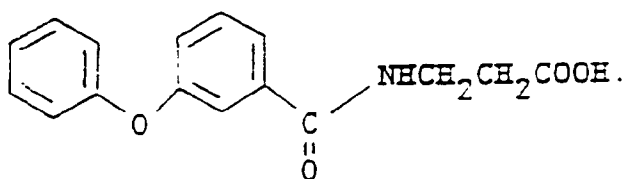
Z_1 is $COOH$, SO_3H , or PO_3H_2 .

12. The method of Claim 11 wherein d is 1, T_1 is a bond, R_{10} is H, W_1 is CH_2CH_2 , and Z_1 is SO_3H .
13. The use of a compound of the formula:

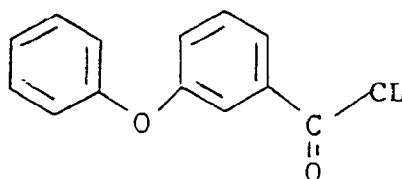


in an in-vitro immunoassay.

14. A process for the preparation of a compound of the formula:

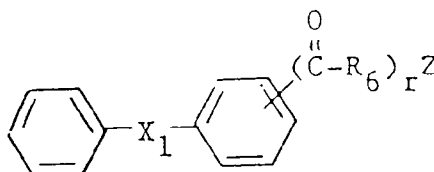


which comprises reacting an acid chloride of formula



with β -alanine.

15. A composition comprising a sample to be analyzed and the compound of formula:



wherein

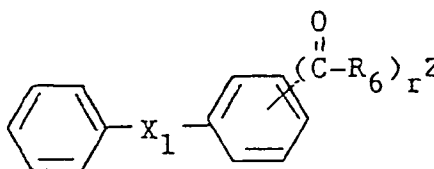
R_6 is $\text{TN}(\text{R}_7)\text{W}$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R_7 is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1; Z is COOH , SO_3H , or PO_3H_2 ; and

X_1 is O, NR_8 , $\text{C}=\text{O}$, $\text{S}(\text{O})_t$, $\text{C}(\text{R}_8)_2$ wherein

R_8 are independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms and t is 0 to 2.

16. The composition of Claim 15 comprising a sample to be analyzed and the compound of Claims 14 or 15.

17. A kit for conducting an assay comprising in packaged combination (a) the compound of formula:



wherein

R_6 is $\text{TN}(\text{R}_7)\text{W}$ wherein T and W are independently a bond or linear or branched chains comprised of 1 to 4 carbon atoms and 0 to 1 oxygen atoms; R_7 is H, or alkyl of 1 to 5 carbon atoms; r is zero or 1; Z is COOH , SO_3H , or PO_3H_2 ; and

X_1 is O, NR_8 , $\text{C}=\text{O}$, $\text{S}(\text{O})_t$, $\text{C}(\text{R}_8)_2$ wherein

R_8 are independently selected from the group consisting of hydrogen and lower alkyl of from 1 to 5 carbon atoms and t is 0 to 2, and (b) reagents for conducting said assay.

18. The kit of Claim 17 for conducting an assay comprising in packaged combination (a) the compound of Claim 14 or 15 and (b) reagents for conducting said assay.

5 Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

1. Verfahren zur Inaktivierung von störend einwirkenden bindenden Proteinen in einem Assay für ein Mitglied eines spezifischen Bindungspaares (sbp), umfassend das Einschließen in einem Assaymedium, das eine Probe enthält, von der man vermutet, daß sie das sbp-Mitglied enthält, einer wasserlöslichen Verbindung der Formel



in der:

20 X aus der Gruppe ausgewählt ist, die aus O, NH, P, N, C, S und CH₂ besteht, worin die ungesättigten Valenzen von N, C, P und S durch Einzel- oder Doppelbindungen zu o-, s- oder C-Atomen hin abgesättigt sein können, welche Gruppen mit einem Molekulargewicht von 14 bis 200 umfassen, wobei die Gruppen Atome aufweisen, die unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff, Kohlenstoff, Stickstoff, Sauerstoff und Schwefel besteht;

25 die Gruppen R unabhängig Gruppen mit 1 bis 12 Atomen, außer Wasserstoff, sind, die eine Kette von 1 bis 10 Atomen, außer Wasserstoff, einschließen können oder miteinander oder mit X zusammengekommen werden können, um einen nicht-benzoiden Ring mit 5 bis 8 Atomen, außer Wasserstoff, zu bilden, wobei die Atome, auf die im ganzen obigen Bezug genommen wurde, unabhängig aus der Gruppe ausgewählt sind, die aus Halogen, Kohlenstoff, Stickstoff, Sauerstoff, Phosphor und Schwefel besteht, worin mindestens ein R eine Einheit enthält, die Wasserlöslichkeit verleiht; und m eine Zahl zwischen 0 und 5 ist, mit der Maßgabe, daß, wenn das sbp-Mitglied oder dessen sbp-Partner zwei Phenylgruppen aufweist, die durch ein gemeinsames Atom verknüpft sind, diese Verbindung eine Anzahl von von Wasserstoff verschiedenen Gruppen an die Phenylgruppen und an das Atom gebunden aufweist, die sich um mindestens zwei von der Anzahl derartiger Nicht-Wasserstoff-Gruppen an dem sbp-Mitglied unterscheiden, und, wenn das sbp-Mitglied oder dessen sbp-Partner zwei Phenylgruppen an ein gemeinsames Atom geknüpft aufweist und das bindende Protein kein Antikörper ist, die Verbindung nur eine von Wasserstoff verschiedene Gruppe aufweist, die an eine Phenylgruppe oder das gemeinsame Atom geknüpft ist.

2. Verfahren nach Anspruch 1, in dem das bindende Protein ein Antikörper ist.
3. Verfahren zum Blockieren der Bindung durch störend einwirkende bindende Proteine in einem Immunassay für einen Liganden, umfassend das Einschließen in einem Medium, von dem man annimmt, daß es einen Liganden und das komplementäre bindende Protein enthält, einer wasserlöslichen Verbindung der folgenden Formel:



in der:

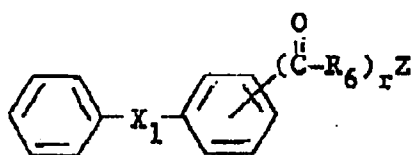
50 X' aus der Gruppe ausgewählt ist, die aus O, NR₄, S(O)_y, C=O und C(R₄)₂ besteht, worin R₄ unabhängig aus der Gruppe ausgewählt ist, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, oder worin R₄ zusammengekommen werden kann, um einen Ring mit 5 bis 8 Atomen, außer Wasserstoff, zu bilden, wobei die Atome unabhängig aus der Gruppe ausgewählt sind, die aus Kohlenstoff, Stickstoff, Sauerstoff und Schwefel besteht, und y eine Zahl von 0 bis 2 ist, und

die R₅ unabhängig aus den Gruppen ausgewählt sind, die 1 bis 12 Atome außer Wasserstoff umfassen, wobei die Atome unabhängig aus der Gruppe ausgewählt sind, die aus Halogen, Kohlenstoff,

Stickstoff, Sauerstoff und Schwefel besteht, wobei die Gruppe eine Einheit einschließt, die Wasserlöslichkeit verleiht,

mit der Maßgabe, daß, wenn der Ligand zwei Phenylgruppen aufweist, welche durch eine Kette verknüpft sind, die die Länge eines Atoms aufweist, die Verbindung eine Anzahl von von Wasserstoff verschiedenen Gruppen, die an die Phenylgruppen und das Atom geknüpft sind, aufweist, welche sich um mindestens zwei von der Anzahl derartiger Gruppen an dem Liganden unterscheidet, und, wenn der Ligand zwei Phenylgruppen aufweist, die an ein gemeinsames Atom geknüpft sind, und das bindende Protein kein Antikörper ist, die Verbindung nur eine von Wasserstoff verschiedene Gruppe aufweist, die an eine Phenylgruppe oder das gemeinsame Atom geknüpft ist.

4. Verfahren nach Anspruch 3, in dem das bindende Protein ein Antikörper ist.
5. Verfahren nach Anspruch 3, in dem das bindende Protein Thyroxin-bindendes Globulin ist.
6. Verfahren nach Anspruch 3, in dem die Verbindung die Formel:



aufweist, in der

R_6 TN(R_7)W ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen; R_7 H oder Alkyl mit 1 bis 5 Kohlenstoffatomen ist; r 0 oder 1 ist;

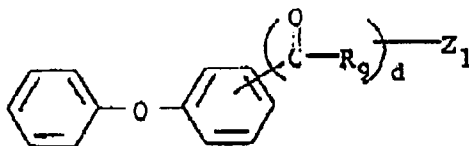
Z COOH, SO_3H oder PO_3H_2 ist; und

X_1 O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ ist, worin die R_8 unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl besteht, und t 0 bis 2 ist.

7. Verfahren nach Anspruch 6, in dem r 0 ist, X_1 O ist und Z COOH ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2 ist, Z SO_3H ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2 ist, Z COOH ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W CH_2 ist, Z COOH ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W $CH(CH_3)$ ist, Z COOH ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W CH_2CH_2 ist; Z SO_3H ist und X_1 CH_2 ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2CH_2 ist, Z SO_3H ist und X_1 O ist, oder r 1 ist, T eine Bindung ist, R_7 H ist, W OCH_2 ist, Z COOH ist und X_1 O ist.

8. Verfahren nach Anspruch 3, in dem es sich bei dem Liganden um ein Thyroid-Hormon, Phenobarbital, Diphenylhydantoin, Tetrahydrocannabinol, ein Benzodiazepin oder Metaboliten derselben handelt.

9. Verfahren zum Verringern einer störenden Einwirkung durch Fenoprofen und dessen Metaboliten in einem Analyten-bindenden Assay, umfassend das Inkontaktbringen des bindenden Proteins, das beim Testen einer Probe verwendet wird, von der man annimmt, daß sie den Analyten enthält, mit einer wirksamen Menge einer Verbindung der Formel:



in der

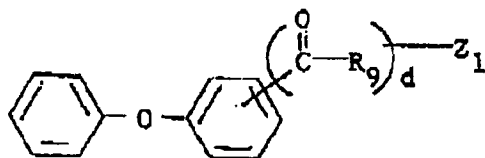
R_9 T₁N(R_{10})W₁ ist, worin T₁ und W₁ unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, und R_{10} H oder Niederalkyl mit 1 bis 5 Kohlenstoffatomen ist, d 0 oder 1 ist, und

Z₁ COOH, SO_3H oder PO_3H_2 ist.

10. Verfahren nach Anspruch 9, in dem d 1 ist, T₁ eine Bindung ist, R₁₀ H ist, W₁ CH₂CH₂ ist und Z₁ SO₃H ist.

11. Verfahren zum Freisetzen von Thyroid-Hormonen aus Thyroxin-bindendem Globulin in einer zu analysierenden Probe, umfassend das Inkontaktbringen der Probe mit einer wirksamen Menge einer Verbindung der Formel:

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in der

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R₉ T₁N(R₇)W₁ ist, worin T₁ und W₁ unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, und R₇ H oder Niederalkyl mit 1 bis 5 Kohlenstoffatomen ist, d 0 oder 1 ist und

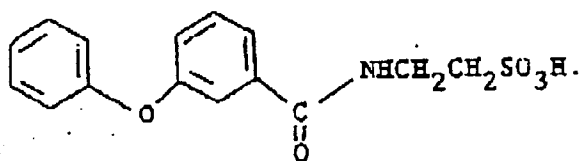
Z₁ COOH, SO₃H oder PO₃H₂ ist.

20

12. Verfahren nach Anspruch 11, in dem d 1 ist, T₁ eine Bindung ist, R₁₀ H ist, W₁ CH₂CH₂ ist und Z₁ SO₃H ist.

13. Verwendung einer Verbindung der Formel:

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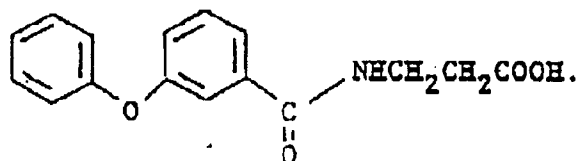


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in einem in-vitro-Immunassay.

14. Verbindung der Formel

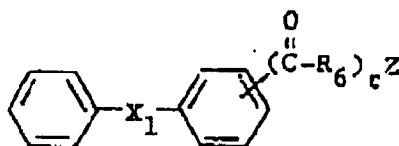
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15. Zusammensetzung, umfassend eine zu analysierende Probe und die Verbindung der Formel:

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in der

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R₆ TN(R₇)W ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen; R₇ H oder Alkyl mit 1 bis 5 Kohlenstoffatomen ist; r null oder 1 ist;

Z COOH, SO₃H oder PO₃H₂ ist; und

X₁ O, NR₈, C=O, S(O)_t, C(R₈)₂ ist, worin die R₈ unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, und t 0 bis 2 ist.

16. Zusammensetzung nach Anspruch 15, umfassend eine zu analysierende Probe und die Verbindung nach den Ansprüchen 14 oder 15.

5 17. Testsatz zum Durchführen eines Assays, in abgepackter Kombination umfassend (a) die Verbindung der Formel:



worin

15 R_6 TN(R_7)W ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, R_7 H oder Alkyl mit 1 bis 5 Kohlenstoffatomen ist; r null oder 1 ist;

Z COOH, SO_3H oder PO_3H_2 ist; und

20 X_1 O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$ ist, worin die R_8 unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, und t 0 bis 2 ist, und (b) Reagenzien zum Durchführen des Assays.

18. Testsatz nach Anspruch 17 zum Durchführen eines Assays, in abgepackter Kombination umfassend (a) die Verbindung nach Anspruch 14 oder 15 und (b) Reagenzien zum Durchführen des Assays.

25 Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Inaktivierung von störend einwirkenden bindenden Proteinen in einem Assay für ein Mitglied eines spezifischen Bindungspaares (sbp), umfassend das Einschließen in einem Assaymedium, das eine Probe enthält, von der man vermutet, daß sie das sbp-Mitglied enthält, einer wasserlöslichen Verbindung der Formel

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in der:

40 X aus der Gruppe ausgewählt ist, die aus O, NH, P, N, C, S und CH_2 besteht, worin die ungesättigten Valenzen von N, C, P und S durch Einzel- oder Doppelbindungen zu O-, S- oder C-Atomen hin abgesättigt sein können, welche Gruppen mit einem Molekulargewicht von 14 bis 200 umfassen, wobei die Gruppen Atome aufweisen, die unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff, Kohlenstoff, Stickstoff, Sauerstoff und Schwefel besteht;

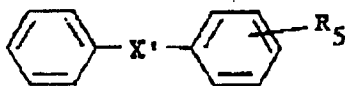
45 die Gruppen R unabhängig Gruppen mit 1 bis 12 Atomen, außer Wasserstoff, sind, die eine Kette von 1 bis 10 Atomen, außer Wasserstoff, einschließen können oder miteinander oder mit X zusammengekommen werden können, um einen nicht-benzoiden Ring mit 5 bis 8 Atomen, außer Wasserstoff, zu bilden, wobei die Atome, auf die im ganzen obigen Bezug genommen wurde, unabhängig aus der Gruppe ausgewählt sind, die aus Halogen, Kohlenstoff, Stickstoff, Sauerstoff, Phosphor und Schwefel besteht, worin mindestens ein R eine Einheit enthält, die Wasserlöslichkeit verleiht; und m eine Zahl zwischen 0 und 5 ist, mit der Maßgabe, daß, wenn das sbp-Mitglied oder dessen sbp-Partner zwei Phenylgruppen aufweist, die durch ein gemeinsames Atom verknüpft sind, diese Verbindung eine Anzahl von von Wasserstoff verschiedenen Gruppen an die Phenylgruppen und an das Atom gebunden aufweist, die sich um mindestens zwei von der Anzahl derartiger Nicht-Wasserstoff-Gruppen an dem sbp-Mitglied unterscheidet, und, wenn das sbp-Mitglied oder dessen sbp-Partner zwei Phenylgruppen an ein gemeinsames Atom geknüpft aufweist und das bindende Protein kein Antikörper ist, die Verbindung nur eine von Wasserstoff verschiedene Gruppe aufweist, die an eine Phenylgruppe oder das gemeinsame Atom geknüpft ist.

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2. Verfahren nach Anspruch 1, in dem das bindende Protein ein Antikörper ist.

3. Verfahren zum Blockieren der Bindung durch störend einwirkende bindende Proteine in einem Immunassay für einen Liganden, umfassend das Einschließen in einem Medium, von dem man annimmt, daß es einen Liganden und das komplementäre bindende Protein enthält, einer wasserlöslichen Verbindung der folgenden Formel:



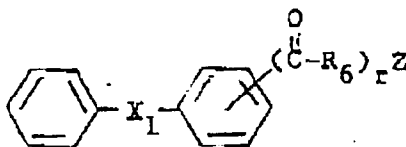
in der:

X' aus der Gruppe ausgewählt ist, die aus O, NR_4 , $S(O)_y$, $C=O$ und $C(R_4)_2$ besteht, worin R_4 unabhängig aus der Gruppe ausgewählt ist, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, oder worin R_4 zusammengefasst werden kann, um einen Ring mit 5 bis 8 Atomen, außer Wasserstoff, zu bilden, wobei die Atome unabhängig aus der Gruppe ausgewählt sind, die aus Kohlenstoff, Stickstoff, Sauerstoff und Schwefel besteht, und y eine Zahl von 0 bis 2 ist, und

die R_5 unabhängig aus den Gruppen ausgewählt sind, die 1 bis 12 Atome außer Wasserstoff umfassen, wobei die Atome unabhängig aus der Gruppe ausgewählt sind, die aus Halogen, Kohlenstoff, Stickstoff, Sauerstoff und Schwefel besteht, wobei die Gruppe eine Einheit einschließt, die Wasserlöslichkeit verleiht,

mit der Maßgabe, daß, wenn der Ligand zwei Phenylgruppen aufweist, welche durch eine Kette verknüpft sind, die die Länge eines Atoms aufweist, die Verbindung eine Anzahl von von Wasserstoff verschiedenen Gruppen, die an die Phenylgruppen und das Atom geknüpft sind, aufweist, welche sich um mindestens zwei von der Anzahl derartiger Gruppen an dem Liganden unterscheidet, und, wenn der Ligand zwei Phenylgruppen aufweist, die an ein gemeinsames Atom geknüpft sind, und das bindende Protein kein Antikörper ist, die Verbindung nur eine von Wasserstoff verschiedene Gruppe aufweist, die an eine Phenylgruppe oder das gemeinsame Atom geknüpft ist.

4. Verfahren nach Anspruch 3, in dem das bindende Protein ein Antikörper ist.
5. Verfahren nach Anspruch 3, in dem das bindende Protein Thyroxin-bindendes Globulin ist,
6. Verfahren nach Anspruch 3, in dem die Verbindung die Formel:



aufweist, in der

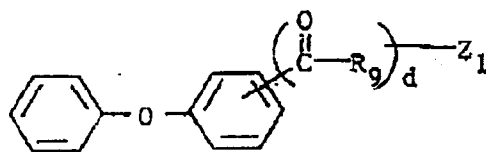
R_6 $TN(R_7)W$ ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen; R_7 oder Alkyl mit 1 bis 5 Kohlenstoffatome ist; r 0 oder 1 ist;

Z $COOH$, SO_3H oder PO_3H_2 ist, und

X_1 O, NR_5 , $C=O$, $S(O)_t$, $C(R_8)_2$ ist, worin die R_8 unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl besteht, und t 0 bis 2 ist.

7. Verfahren nach Anspruch 6, in dem r 0 ist, X_1 O ist und Z $COOH$ ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2 ist, Z SO_3H ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2 ist, Z $COOH$ ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W CH_2 ist, Z $COOH$ ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W $CH(CH_3)$ ist, Z $COOH$ ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W CH_2CH_2 ist; Z SO_3H ist und X_1 CH_2 ist; oder r 1 ist, T eine Bindung ist, R_7 CH_3 ist, W CH_2CH_2 ist, Z SO_3H ist und X_1 O ist; oder r 1 ist, T eine Bindung ist, R_7 H ist, W OCH_2 ist, Z $COOH$ ist und X_1 O ist.
8. Verfahren nach Anspruch 3, in dem es sich bei dem Liganden um ein Thyroid-Hormon, Phenobarbital, Diphenylhydantoin, Tetrahydrocannabinol, ein Benzodiazepin oder Metaboliten derselben handelt.

9. Verfahren zum Verringern einer störenden Einwirkung durch Fenoprofen und dessen Metaboliten in einem Analyten-bindenden Assay, umfassend das Inkontaktbringen des bindenden Proteins, das beim Testen einer Probe verwendet wird, von der man annimmt, daß sie den Analyten enthält, mit einer wirksamen Menge einer Verbindung der Formel:

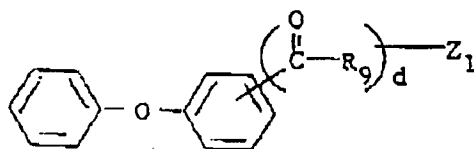


in der

R_9 $T_1N(R_{10})W_1$ ist, worin T_1 und W_1 unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, und R_{10} H oder Niederalkyl mit 1 bis 5 Kohlenstoffatomen ist, d 0 oder 1 ist, und Z_1 $COOH$, SO_3H oder PO_3H_2 ist.

10. Verfahren nach Anspruch 9, in dem d 1 ist, T_1 eine Bindung ist, R_{10} H ist, W_1 CH_2CH_2 ist und Z_1 SO_3H ist.

11. Verfahren zum Freisetzen von Thyroid-Hormonen aus Thyroxin-bindendem Globulin in einer zu analysierenden Probe, umfassend das Inkontaktbringen der Probe mit einer wirksamen Menge einer Verbindung der Formel:

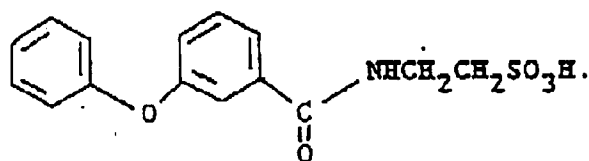


in der

R_9 $T_1N(R_7)W_1$ ist, worin T_1 und W_1 unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, und R_7 H oder Niederalkyl mit 1 bis 5 Kohlenstoffatomen ist, d 0 oder 1 ist und Z_1 $COOH$, SO_3H oder PO_3H_2 ist.

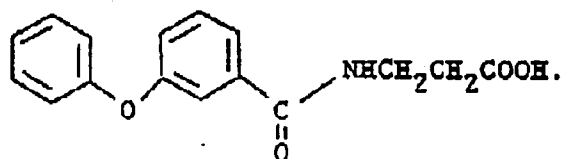
12. Verfahren nach Anspruch 11, in dem d 1 ist, T_1 eine Bindung ist, R_{10} H ist, W_1 CH_2CH_2 ist und Z_1 SO_3H ist.

13. Verwendung einer Verbindung der Formel:

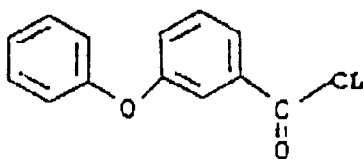


in einem in-vitro-Immunassay.

14. Verfahren zur Herstellung einer Verbindung der Formel:

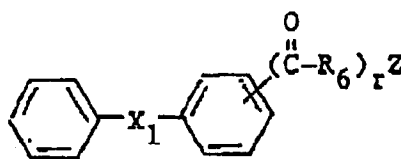


umfassend das Umsetzen eines Säurechlorides der Formel



mit β -Alanin.

15. Zusammensetzung, umfassend eine zu analysierende Probe und die Verbindung der Formel:



in der

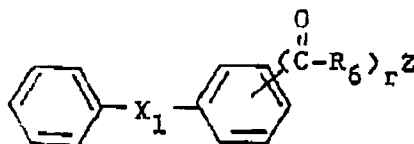
R_6 TN(R_7)W ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen; R_7 H oder Alkyl mit 1 bis 5 Kohlenstoffatomen ist; r null oder 1 ist;

Z COOH, SO_3H oder PO_3H_2 ist; und

X_1 O, NR_8 , C=O, S(O)_t , $\text{C(R}_8)_2$ ist, worin die R_8 unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, und t 0 bis 2 ist.

16. Zusammensetzung nach Anspruch 15, umfassend eine zu analysierende Probe und die Verbindung nach den Ansprüchen 14 oder 15.

17. Testsatz zum Durchführen eines Assays, in abgepackter Kombination umfassend (a) die Verbindung der Formel:



worin

R_6 TN(R_7)W ist, worin T und W unabhängig eine Bindung oder lineare oder verzweigte Ketten sind, die 1 bis 4 Kohlenstoffatome und 0 bis 1 Sauerstoffatome umfassen, R_7 H oder Alkyl mit 1 bis 5 Kohlenstoffatomen ist; r null oder 1 ist;

Z COOH, SO_3H oder PO_3H_2 ist; und

X_1 O, NR_8 , C=O, S(O)_t , $\text{C(R}_8)_2$ ist, worin die R_8 unabhängig aus der Gruppe ausgewählt sind, die aus Wasserstoff und Niederalkyl mit 1 bis 5 Kohlenstoffatomen besteht, und t 0 bis 2 ist, und (b) Reagenzien zum Durchführen des Assays.

18. Testsatz nach Anspruch 17 zum Durchführen eines Assays, in abgepackter Kombination umfassend (a) die Verbindung nach Anspruch 14 oder 15 und (b) Reagenzien zum Durchführen des Assays.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

1. Procédé pour inactiver les protéines de liaison gênantes dans un dosage d'un membre de couple de liaison

spécifique (spb), qui comprend l'incorporation dans un milieu de dosage contenant un échantillon supposé contenir ledit membre de spb d'un composé soluble dans l'eau de formule:

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dans laquelle:

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X est choisi dans le groupe constitué de O, NH, P, N, C, S et CH₂ dans lesquels les valences libres de N, C, P et de S peuvent être occupées par des liaisons simples ou doubles d'atomes de O, de S ou de C qui comprennent des groupes de poids moléculaire de 14 à 200, lesdits groupes ayant des atomes indépendamment choisis dans le groupe constitué d'atomes d'hydrogène, de carbone, d'azote, d'oxygène et de soufre;

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les groupes R sont indépendamment des groupes de 1 à 12 atomes autres que l'atome d'hydrogène qui peuvent comprendre une chaîne de 1 à 10 atomes autres que l'atome d'hydrogène ou peuvent former ensemble ou conjointement avec X un cycle qui n'est pas du type benzénique possédant de cinq à huit atomes autres que l'atome d'hydrogène, lesquels atomes dont il a été fait mention ci-dessus sont indépendamment choisis dans le groupe constitué d'atomes d'halogène, de carbone, d'azote, d'oxygène, de phosphore et de soufre dans lesquels au moins un groupe R contient une portion conférant une solubilité à la molécule dans l'eau; et m est un nombre compris entre 0 et 5, à la condition que, lorsque ledit membre de spb ou son partenaire de spb possède deux groupes phényle liés entre eux par un atome commun, ledit composé comporte plusieurs groupes autres que l'atome d'hydrogène attachés auxdits groupes phényle et la différence entre le nombre desdits atomes et le nombre de tels groupes différents de l'atome d'hydrogène sur ledit membre de spb étant au moins égale à deux et lorsque ledit membre de spb ou son partenaire spb possède deux groupes phényle liés entre eux par un atome commun et que la protéine de liaison n'est pas un anticorps, le composé possède un seul groupe autre que l'atome d'hydrogène lié à un groupe phényle ou à l'atome commun.

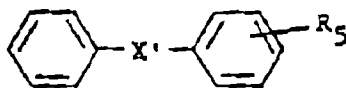
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30 2. Procédé selon la revendication 1 dans lequel la protéine de liaison est un anticorps.

3. Procédé pour inhiber la liaison de protéines de liaison gênantes dans le dosage immunologique d'un ligand, qui comprend l'incorporation dans le milieu supposé contenir un ligand et ladite protéine de liaison complémentaire, un composé soluble dans l'eau répondant à la formule suivante:

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dans laquelle X' est choisi dans le groupe constitué de O, NR₄, S(O)_y, C=O, et C(R₄)₂, R₄ étant choisi indépendamment dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 5 atomes de carbone ou R₄ pouvant être pris conjointement pour former un cycle ayant de cinq à huit atomes autres que l'atome d'hydrogène, lesquels atomes sont indépendamment choisis dans le groupe constitué d'atomes de carbone, d'azote, d'oxygène et de soufre et y est un nombre allant de 0 à 2 et,

45

R₅ est indépendamment choisi parmi les groupes comprenant de un à douze atomes de carbone autres que l'atome d'hydrogène, lesquels atomes sont indépendamment choisis dans le groupe constitué d'atomes d'halogène, de carbone, d'azote, d'oxygène et de soufre, ledit groupe comprenant une portion qui confère une solubilité à la molécule dans l'eau,

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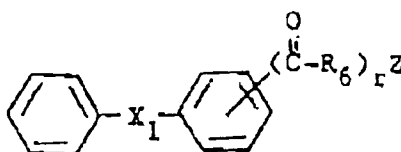
à la condition que, lorsque ledit ligand possède deux groupes phényle liés entre eux par une chaîne comportant un seul atome, ledit composé comporte plusieurs groupes autres que l'atome d'hydrogène attachés auxdits groupes phényle et la différence entre ledit atome et le nombre de tels groupes sur ledit ligand est au moins égale à deux et, lorsque le ligand possède deux groupes phényle liés entre eux par un atome commun et que la protéine de liaison n'est pas un anticorps, ledit composé possède un seul groupe autre que l'atome d'hydrogène lié à un groupe phényle ou à l'atome commun.

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4. Procédé selon la revendication 3 dans lequel ladite protéine de liaison est un anticorps.

5. Procédé selon la revendication 3 dans lequel ladite protéine de liaison est une thyroglobuline.

6. Procédé selon la revendication 3 dans lequel ledit composé est de formule:



dans laquelle:

R_6 est $\text{TN}(\text{R}_7)\text{W}$ dans lequel T et W sont indépendamment une liaison ou une chaîne linéaire ou ramifiée comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

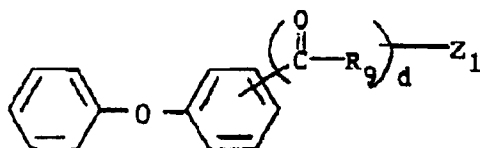
Z est COOH , SO_3H ou PO_3H_2 ; et

X_1 est O, NR_8 , $\text{C}=\text{O}$, $\text{S}(\text{O})_t$, $\text{C}(\text{R}_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur et t varie de 0 à 2.

7. Procédé selon la revendication 6 dans lequel r est égal à zéro, X_1 est O, et Z est COOH ; ou r est égal à 1, T est une liaison, R_7 est CH_3 , W est CH_2 , Z est SO_3H , et X_1 est O, ou r est égal à 1, T est une liaison, R_7 est CH_3 , W est CH_2 , Z est COOH , et X_1 est O; ou r est égal à 1, T est une liaison, R_7 est H, W est CH_2 , Z est COOH , et X_1 est égal à 0; ou r est égal à 1, T est une liaison, R_7 est H, W est $\text{CH}(\text{CH}_3)$, Z est COOH , et X_1 est égal à 0; ou r est égal à 1, T est une liaison, R_7 est H, W est CH_2CH_2 , Z est SO_3H , et X_1 est CH_2 ; ou r est égal à 1, T est une liaison, R_7 est CH_3 , W est CH_2CH_2 , Z est SO_3H , et X_1 est O; ou r est égal à 1, T est une liaison, R_7 est H, W est OCH_2 , Z est COOH , et X_1 est O.

8. Procédé selon la revendication 3 dans lequel ledit ligand est une hormone thyroïdienne, le phénobarbital, la phénytoïne, le tétrahydrocannabinol, une benzodiazépine, ou des métabolites de ceux-ci.

9. Procédé pour réduire l'interférence par le fénopropfen et ses métabolites dans un dosage de liaison à une substance à doser qui comprend la mise en contact de la protéine de liaison utilisée dans le dosage d'un échantillon supposé contenir ladite substance à doser avec une quantité efficace d'un composé de formule:



dans laquelle

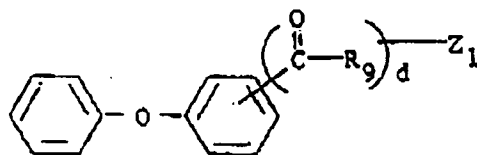
R_9 est $\text{T}_1\text{N}(\text{R}_{10})\text{W}_1$ dans lequel T_1 et W_1 sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène et R_{10} est H ou un groupe alkyle inférieur ayant de 1 à 5 atomes de carbone, d est égal à 0 ou à 1, et

Z_1 est COOH , SO_3H , ou PO_3H_2 .

10. Procédé selon la revendication 9 dans lequel d est égal à 1, T_1 est une liaison, R_{10} est H, W_1 est CH_2CH_2 et Z_1 est SO_3H .

11. Procédé pour dissocier les hormones thyroïdiennes de la thyroglobuline dans un échantillon à doser, lequel procédé comprend la mise en contact dudit échantillon avec une quantité efficace d'un composé de formule:

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dans laquelle:

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R_9 est $T_1N(R_7)W_1$ dans lequel T_1 et W_1 sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène et R_7 est H ou un groupe alkyle inférieur ayant de 1 à 5 atomes de carbone, d est égal à 0 ou à 1, et

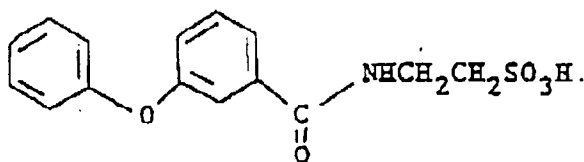
Z_1 est $COOH$, SO_3H ou PO_3H_2 .

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12. Procédé selon la revendication 11 dans lequel d est égal à 1, T_1 est une liaison, R_{10} est H, W_1 est CH_2CH_2 , et Z_1 est SO_3H .

13. Utilisation d'un composé de formule:

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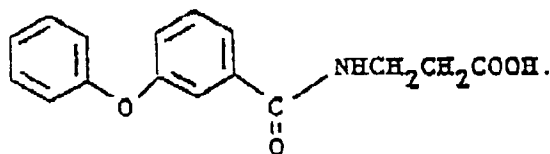


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dans un dosage immunologique *in vitro*.

14. Composé de formule:

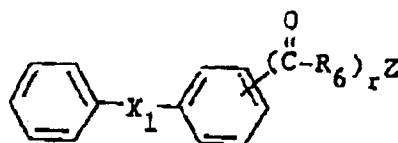
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15. Composition comprenant un échantillon à doser et le composé de formule:

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dans laquelle

R_6 est $TN(R_7)W$ dans lequel T et W sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

Z est $COOH$, SO_3H ou PO_3H_2 ; et

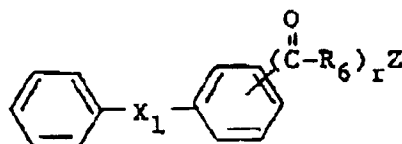
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X_1 est O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 15 atomes de carbone et t varie de 0 à 2.

55

16. Composition selon la revendication 15 comprenant un échantillon à doser et le composé selon la revendication 14 ou 15.

17. Trousse pour réaliser un dosage comprenant dans le même coffret (a) le composé de formule:



dans laquelle

R_6 est $TN(R_7)W$ dans lequel T et W sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

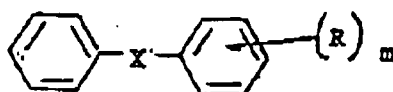
Z est $COOH$, SO_3H ou PO_3H_2 ; et

X_1 est O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 5 atomes de carbone et t varie de 0 à 2, et (b) les réactifs pour réaliser ledit dosage.

18. Trousse selon la revendication 17 pour réaliser un dosage comprenant dans le même coffret (a) le composé selon la revendication 14 ou 15 et (b) les réactifs pour réaliser ledit dosage.

Revendications pour l'Etat contractant suivant : ES

1. Procédé pour inactiver les protéines de liaison gênantes dans un dosage d'un membre de couple de liaison spécifique (spb), qui comprend l'incorporation dans un milieu de dosage contenant un échantillon supposé contenir ledit membre de spb d'un composé soluble dans l'eau de formule:

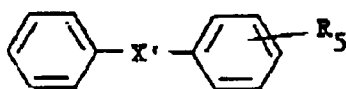


dans laquelle:

X est choisi dans le groupe constitué de O, NH, P, N, C, S et CH_2 dans lesquels les valences libres de N, C, P et de S peuvent être occupées par des liaisons simples ou doubles d'atomes de O, de S ou de C qui comprennent des groupes de poids moléculaire de 14 à 200, lesdits groupes ayant des atomes indépendamment choisis dans le groupe constitué d'atomes d'hydrogène, de carbone, d'azote, d'oxygène et de soufre;

les groupes R sont indépendamment des groupes de 1 à 12 atomes autres que l'atome d'hydrogène qui peuvent comprendre une chaîne de 1 à 10 atomes autres que l'atome d'hydrogène ou peuvent former ensemble ou conjointement avec X un cycle qui n'est pas du type benzénique possédant de cinq à huit atomes autres que l'atome d'hydrogène, lesquels atomes dont il a été fait mention ci-dessus sont indépendamment choisis dans le groupe constitué d'atomes d'halogène, de carbone, d'azote, d'oxygène, de phosphore et de soufre dans lesquels au moins un groupe R contient une portion conférant une solubilité à la molécule dans l'eau; et m est un nombre compris entre 0 et 5, à la condition que, lorsque ledit membre de spb ou son partenaire de spb possède deux groupes phényle liés entre eux par un atome commun, ledit composé comporte plusieurs groupes autres que l'atome d'hydrogène attachés auxdits groupes phényle et la différence entre le nombre desdits atomes et le nombre de tels groupes différents de l'atome d'hydrogène sur ledit membre de spb étant au moins égale à deux et lorsque ledit membre de spb ou son partenaire spb possède deux groupes phényle liés entre eux par un atome commun et que la protéine de liaison n'est pas un anticorps, le composé possède un seul groupe autre que l'atome d'hydrogène lié à un groupe phényle ou à l'atome commun.

2. Procédé selon la revendication 1 dans lequel la protéine de liaison est un anticorps.
3. Procédé pour inhiber la liaison de protéines de liaison gênantes dans le dosage immunologique d'un ligand, qui comprend l'incorporation dans le milieu supposé contenir un ligand et ladite protéine de liaison complémentaire, un composé soluble dans l'eau répondant à la formule suivante:



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dans laquelle X' est choisi dans le groupe constitué de O, NR_4 , $S(O)_y$, $C=O$, et $C(R_4)_2$, R_4 étant choisi indépendamment dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 5 atomes de carbone ou R_4 pouvant être pris conjointement pour former un cycle ayant de cinq à huit atomes autres que l'atome d'hydrogène, lesquels atomes sont indépendamment choisis dans le groupe constitué d'atomes de carbone, d'azote, d'oxygène et de soufre et y est un nombre allant de 0 à 2 et,

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R_5 est indépendamment choisi parmi les groupes comprenant de un à douze atomes de carbone autres que l'atome d'hydrogène, lesquels atomes sont indépendamment choisis dans le groupe constitué d'atomes d'halogène, de carbone, d'azote, d'oxygène et de soufre, ledit groupe comprenant une portion qui confère une solubilité à la molécule dans l'eau,

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à la condition que, lorsque ledit ligand possède deux groupes phényle liés entre eux par une chaîne comportant un seul atome, ledit composé comporte plusieurs groupes autres que l'atome d'hydrogène attachés auxdits groupes phényle et la différence entre ledit atome et le nombre de tels groupes sur ledit ligand est au moins égale à deux et, lorsque le ligand possède deux groupes phényle liés entre eux par un atome commun et que la protéine de liaison n'est pas un anticorps, ledit composé possède un seul groupe autre que l'atome d'hydrogène lié à un groupe phényle ou à l'atome commun.

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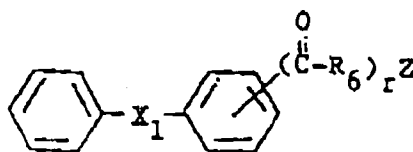
4. Procédé selon la revendication 3 dans lequel ladite protéine de liaison est un anticorps.

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5. Procédé selon la revendication 3 dans lequel ladite protéine de liaison est une thyroglobuline.

6. Procédé selon la revendication 3 dans lequel ledit composé est de formule:

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dans laquelle:

R_6 est $TN(R_7)W$ dans lequel T et W sont indépendamment une liaison ou une chaîne linéaire ou ramifiée comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

Z est $COOH$, SO_3H ou PO_3H_2 ; et

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X_1 est O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur et t varie de 0 à 2.

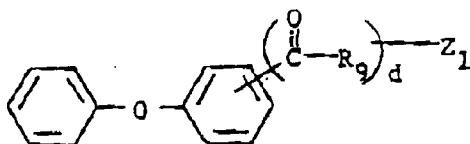
7. Procédé selon la revendication 6 dans lequel r est égal à zéro, X_1 est O, et Z est $COOH$; ou r est égal à 1; T est une liaison, R_7 est CH_3 , W est CH_2 , Z est SO_3H , et X_1 est O, ou r est égal à 1, T est une liaison, R_7 est CH_3 , W est CH_2 , Z est $COOH$, et X_1 est O; ou r est égal à 1, T est une liaison, R_7 est H, W est CH_2 , Z est $COOH$, et X_1 est égal à 0; ou r est égal à 1, T est une liaison, R_7 est H, W est $CH(CH_3)$, Z est $COOH$, et X_1 est égal à O, ou r est égal à 1, T est une liaison, R_7 est H, W est CH_2CH_2 , Z est SO_3H , et X_1 est CH_2 ; ou r est égal à 1, T est une liaison, R_7 est CH_3 , W est CH_2CH_2 , Z est SO_3H , et X_1 est O; ou r est égal à 1, T est une liaison, R_7 est H, W est OCH_2 , Z est $COOH$, et X_1 est O.

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8. Procédé selon la revendication 3 dans lequel ledit ligand est une hormone thyroïdienne, le phénobarbital, la phénytoïne, le tétrahydrocannabinol, une benzodiazépine, ou des métabolites de ceux-ci.

9. Procédé pour réduire l'interférence par le fénoprofen et ses métabolites dans un dosage de liaison à une substance à doser qui comprend la mise en contact de la protéine de liaison utilisée dans le dosage d'un échantillon supposé contenir ladite substance à doser avec une quantité efficace d'un composé de formule:

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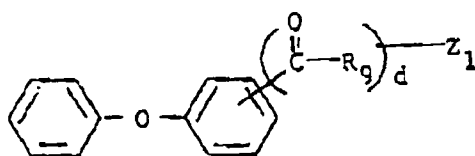
dans laquelle

R_9 est $T_1N(R_{10})W_1$ dans lequel T_1 et W_1 sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène et R_{10} est H ou un groupe alkyle inférieur ayant de 1 à 5 atomes de carbone, d est égal à 0 ou à 1, et

Z_1 est COOH , SO_3H , ou PO_3H_2 .

10. Procédé selon la revendication 9 dans lequel d est égal à 1, T_1 est une liaison, R_{10} est H, W_1 est CH_2CH_2 et Z_1 est SO_3H .

11. Procédé pour dissocier les hormones thyroïdiennes de la thyroglobuline dans un échantillon à doser, lequel procédé comprend la mise en contact dudit échantillon avec une quantité efficace d'un composé de formule:



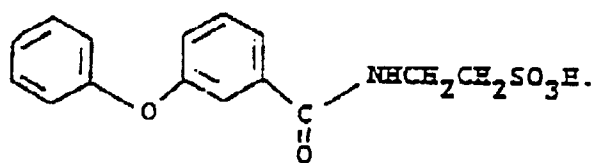
dans laquelle:

R_9 est $T_1N(R_7)W_1$ dans lequel T_1 et W_1 sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène et R_7 est H ou un groupe alkyle inférieur ayant de 1 à 5 atomes de carbone, d est égal à 0 ou à 1, et

Z_1 est COOH , SO_3H ou PO_3H_2 .

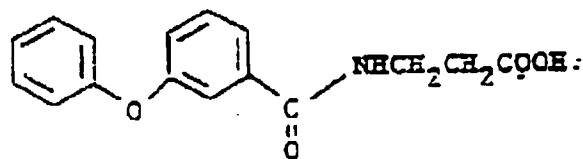
12. Procédé selon la revendication 11 dans lequel d est égal à 1, T_1 est une liaison, R_{10} est H, W_1 est CH_2CH_2 , et Z_1 est SO_3H .

13. Utilisation d'un composé de formule:

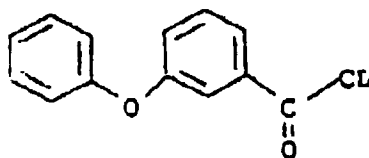


dans un dosage immunologique *in vitro*.

14. Procédé pour la préparation d'un composé de formule:

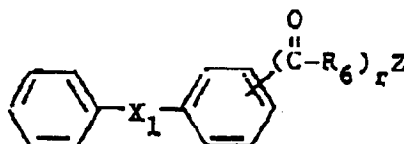


qui comprend la mise en réaction d'un chlorure d'acide de formule



avec une β -alanine.

15. Composition comprenant un échantillon à doser et le composé de formule:



dans laquelle

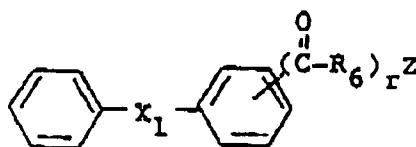
R_6 est $TN(R_7)W$ dans lequel T et W sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

Z est $COOH$, SO_3H ou PO_3H_2 ; et

X_1 est O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 15 atomes de carbone et t varie de 0 à 2.

16. Composition selon la revendication 15 comprenant un échantillon à doser et le composé selon la revendication 14 ou 15.

17. Trousse pour réaliser un dosage comprenant dans le même coffret (a) le composé de formule:



dans laquelle

R_6 est $TN(R_7)W$ dans lequel T et W sont indépendamment une liaison ou des chaînes linéaires ou ramifiées comprenant de 1 à 4 atomes de carbone et de 0 à 1 atome d'oxygène; R_7 est H, ou un groupe alkyle de 1 à 5 atomes de carbone; r est égal à zéro ou à 1;

Z est $COOH$, SO_3H ou PO_3H_2 ; et

X_1 est O, NR_8 , $C=O$, $S(O)_t$, $C(R_8)_2$, dans lesquels les groupes R_8 sont indépendamment choisis dans le groupe constitué de l'atome d'hydrogène et des groupes alkyle inférieur ayant de 1 à 5 atomes de carbone et t varie de 0 à 2, et (b) les réactifs pour réaliser ledit dosage.

18. Trousse selon la revendication 17 pour réaliser un dosage comprenant dans le même coffret (a) le composé selon la revendication 14 ou 15 et (b) les réactifs pour réaliser ledit dosage.